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(54) Title: COMBINATIONS FOR THE TREATMENT OF FUNGAL INFECTIONS

(57) Abstract: The invention features a method for treating or preventing fungal growth by contacting fungal cells with (I) an aromatic diamidine or analog thereof or a compound formula (I); and (ii) an aminopyridine, a quaternary ammonium compound, or a compound of formula (II) or (III) simultaneously or within 14 days of each other in amounts sufficient to reduce or inhibit fungal growth.

# COMBINATIONS FOR THE TREATMENT OF FUNGAL INFECTIONS

## Background of the Invention

The invention relates to the treatment of fungal infections.

In animals, fungal infections (mycoses) may be superficial, sub-cutaneous, or systemic. Fungal infections, especially of the skin and fingernails, are very common in children, but they can affect all age groups. Superficial mycoses include: tinea capitis, an infection of the neck and scalp; tinea corporis, infections to the body; tinea pedis, infections to the foot, also known as athlete's foot; tinea barbae, infections along the beard area, also known as barber's itch; tinea cruris, infections to the groin area; and tinea versicolor and tinea unguium, which involve infection of the nails. Other superficial mycoses include onychomycosis, perionychomycosis, pityriasis versicolor, oral thrush, and candidoses such as vaginal, respiratory tract, biliary, eosophageal, and urinary tract candidoses.

Systemic mycoses occur when spores are inhaled or enter into the body. Examples of systemic infections include mucocutaneous candidosis, chromoblastomycosis, mycetoma, cryptococcosis, aspergillosis, mucormycosis, paracoccidioidomycosis, North American blastomycosis, histoplasmosis, coccidioidomycosis (San Joaquin or valley fever), and sporotrichosis. As with most systemic pathogens, if left untreated, serious life-threatening infections can develop.

The need for novel antifungal treatments is significant, and is especially critical in the medical field. Immunocompromised patients provide perhaps the greatest challenge to modern health care delivery. During the last three decades there has been a dramatic increase in the frequency of fungal infections in these patients (Herbrecht, Eur. J. Haematol., 56:12, 1996; Cox et al., Curr. Opin. Infect. Dis., 6:422, 1993; Fox, ASM News, 59:515, 1993). Deep-seated

mycoses are increasingly observed in patients undergoing organ transplants and in patients receiving aggressive cancer chemotherapy (Alexander et al., Drugs, 54:657, 1997). The most common pathogens associated with invasive fungal infections are the opportunistic yeast, *Candida albicans*, and the filamentous fungus, *Aspergillus fumigatus* (Bow, Br. J. Haematol., 101:1, 1998; Wamock, J. Antimicrob. Chemother., 41:95, 1998). There are an estimated 200,000 patients per year who acquire nosocomial fungal infections (Beck-Sague et al., J. Infect. Dis. 167:1247, 1993). Also adding to the increase in the numbers of fungal infections is the emergence of Acquired Immunodeficiency Syndrome (AIDS) where virtually all patients become affected with some form of mycoses during the course of the disease (Alexander et al., Drugs, 54:657, 1997; Hood et al., J. Antimicrob. Chemother., 37:71, 1996). The most common organisms encountered in these patients are *Cryptococcus neoformans*, *Pneumocystis carinii*, and *C. albicans* (HIV/AIDS Surveillance Report, 1996, 7(2), Year-End Edition; Polis, M. A. et al., AIDS: Biology, Diagnosis, Treatment and Prevention, fourth edition, 1997). New opportunistic fungal pathogens such as *Penicillium marneffei*, *C. krusei*, *C. glabrata*, *Histoplasma capsulatum*, and *Coccidioides immitis* are being reported with regularity in immunocompromised patients throughout the world.

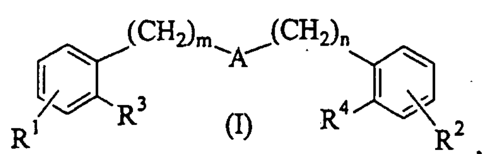
The development of antifungal treatment regimens has been a continuing challenge. Currently available drugs for the treatment of fungal infections include amphotericin B, a macrolide polyene that interacts with fungal membrane sterols, flucytosine, a fluoropyrimidine that interferes with fungal protein and DNA biosynthesis, and a variety of azoles (e.g., ketoconazole, itraconazole, and fluconazole) that inhibit fungal membrane-sterol biosynthesis (Alexander et al., Drugs, 54:657, 1997). Even though amphotericin B has a broad range of activity and is viewed as the "gold standard" of antifungal therapy, its use is limited due to infusion-related reactions and nephrotoxicity (Wamock, J. Antimicrob. Chemother., 41:95,

1998). Flucytosine usage is also limited due to the development of resistant microbes and its narrow spectrum of activity. The widespread use of azoles is causing the emergence of clinically-resistant strains of *Candida spp*. Due to the problems associated with the current treatments, there is an ongoing search for new treatments.

**Summary of the Invention**

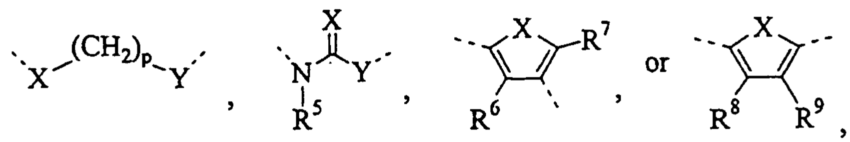
We have discovered that the combination of an antiprotozoal drug, pentamidine, and a diaminopyridine, phenazopyridine, or a quaternary ammonium compound, pentolinium, brings about substantial inhibition of growth of triazole-resistant and triazole-susceptible strains of *C. albicans in vitro*. Pentamidine and phenazopyridine also inhibited growth of two other *Candida* species (*C. krusei* and *C. glabrata)* and *Cryptococcus neoformans* and *Aspergillus*. The combination of pentamidine and pentolinium exhibits fungicidal activity, whereas only fungistatic activity was observed when either drug was used separately. Thus, these combinations can be used to treat fungal infections. Moreover, based on the shared action among aromatic diamidino family members, aminopyridine family members, and quaternary ammonium compound family members, pentamidine and/or phenazopyridine or pentolinium can be replaced by a family member in the combination.

Accordingly, in one aspect, the invention features a method for treating a patient having a fungal infection by administering to the patient:
a first compound having the formula (I):

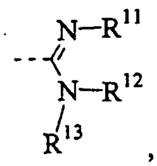


or a pharmaceutically acceptable salt thereof,

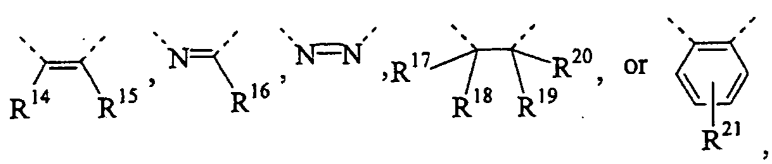
wherein A is



wherein each of X and Y is, independently, O, $NR^{10}$, or S, each of $R^5$ and $R^{10}$ is, independently, H or $C_1$-$C_6$ alkyl, each of $R^6$, $R^7$, $R^8$, and $R^9$ is, independently, H, $C_1$-$C_6$ alkyl, halogen, $C_1$-$C_6$ alkyloxy,$C_6$-$C_{18}$ aryloxy, or $C_6$-$C_{18}$ aryl-$C_1$-$C_6$ alkyloxy, p is an integer between 2 and 6, inclusive, each of m and n is, independently, an integer between 0 and 2, inclusive, each of $R^1$ and $R^2$ is

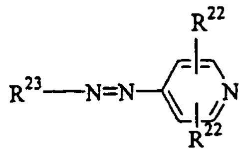


wherein $R^{12}$ is H, $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkyloxy-$C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, or $C_6$-$C_{18}$ aryl, $R^{13}$ is H, $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkyloxy, $C_1$-$C_6$ alkyloxy $C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, carbo($C_1$-$C_6$ alkyloxy), carbo($C_6$-$C_{18}$ aryl $C_1$-$C_6$ alkyloxy), carbo($C_6$-$C_{18}$ aryloxy), or $C_6$-$C_{18}$ aryl, and $R^{11}$ is H, OH, or $C_1$-$C_6$ alkyloxy, or $R^{11}$ and $R^{12}$ together represent



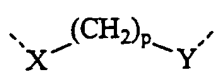
wherein each of $R^{14}$, $R^{15}$, and $R^{16}$ is, independently, H, $C_1$-$C_6$ alkyl, halogen, or trifluoromethyl, each of $R^{17}$, $R^{18}$, $R^{19}$, and $R^{20}$ is, independently, H or $C_1$-$C_6$ alkyl, and $R^{21}$ is H, halogen, trifluoromethyl, $OCF_3$, $NO_2$, $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkyloxy, $C_1$-$C_6$ alkoxy $C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, or $C_6$-$C_{18}$ aryl, each of $R^3$ and

$R^4$ is, independently, H, Cl, Br, OH, $OCH_3$, $OCF_3$, $NO_2$, and $NH_2$, or $R^3$ and $R^4$ together form a single bond; and a second compound having the formula (II):


(II)

wherein each $R^{22}$ is, independently, $NH_2$, H, OH, a halide, $C_{1-10}$ alkyl, $C_{1-10}$ alkoxyalkyl, hydroxyalkyl (wherein the alkyl group has from 1 to 10 carbon atoms), aminoalkyl (wherein the alkyl group has from 1 to 10 carbon atoms), $C_{1-10}$ alkylaminoalkyl, cycloalkyl (wherein the alkyl group has from 1 to 10 carbon atoms), aryl, or $C_{1-10}$ alkylaryl; and $R^{23}$ is $NH_2$, H, OH, a halide, $C_{1-10}$ alkyl, $C_{1-10}$ alkoxyalkyl, hydroxyalkyl (wherein the alkyl group has from 1 to 10 carbon atoms), aminoalkyl (wherein the alkyl group has from 1 to 10 carbon atoms), $C_{1-10}$ alkylaminoalkyl, cycloalkyl (wherein the alkyl group has from 1 to 10 carbon atoms), aryl, or $C_{1-10}$ alkylaryl. The first and second compounds are administered within 10 days of each other, within five days of each other, within 24 hours of each other, within one hour of each other, or are administered simultaneously, in amounts sufficient to treat or inhibit a fungal infection in a patient.

In a related aspect, in the compound of formula (I), A is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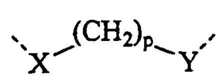
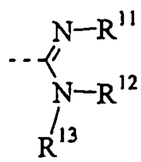
each of X and Y is independently O or NH, p is an integer between 2 and 6, inclusive, and m and n are, independently, integers between 0 and 2, inclusive, wherein the sum of m and n is greater than 0; or A is

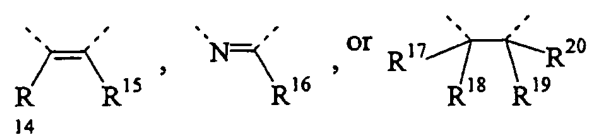

,

each of X and Y is independently O or NH, each of m and n is 0, and each of $R^1$ and $R^2$ is, independently, selected from the group represented by



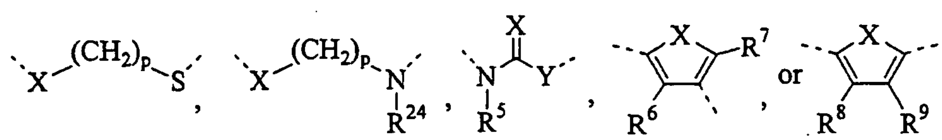
,

wherein $R^{12}$ is $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkoxy $C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, or $C_6$-$C_{18}$ aryl, $R^{13}$ is H, $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkyloxy, $C_1$-$C_6$ alkoxy $C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, carbo($C_1$-$C_6$ alkoxy), carbo($C_6$-$C_{18}$ aryl $C_1$-$C_6$ alkoxy), carbo($C_6$-$C_{18}$ aryloxy), or $C_6$-$C_{18}$ aryl, and $R^{11}$ is H, OH, or $C_1$-$C_6$ alkyloxy, or $R^{11}$ and $R^{12}$ together represent



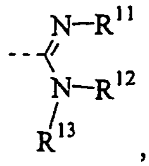
,

wherein each of $R^{14}$, $R^{15}$, and $R^{16}$ is, independently, H, $C_1$-$C_6$ alkyl, halogen, or trifluoromethyl, each of $R^{17}$, $R^{18}$, and $R^{19}$ is, independently, H or $C_1$-$C_6$ alkyl, and $R^{20}$ is $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkyloxy, or trifluoromethyl; or A is

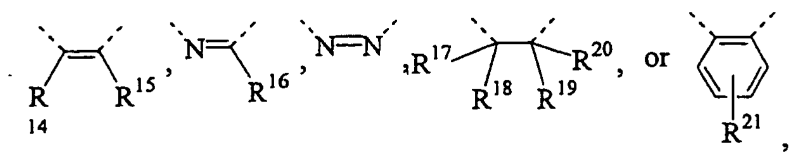


,

each of X and Y is, independently, O, $NR^{10}$, or S, each of $R^5$ and $R^{10}$ is, independently, H or $C_1$-$C_6$ alkyl, each of $R^6$, $R^7$, $R^8$, and $R^9$ is, independently, H, $C_1$-$C_6$ alkyl, halogen, $C_1$-$C_6$ alkyloxy, $C_6$-$C_{18}$ aryloxy, or $C_6$-$C_{18}$ aryl $C_1$-$C_6$ alkyloxy, $R^{24}$ is $C_1$-$C_6$ alkyl, p is an integer between 2 and 6, inclusive, each of m and n is, independently, an integer between 0 and 2, inclusive, each of $R^1$ and $R^2$ is, independently, selected from the group represented by

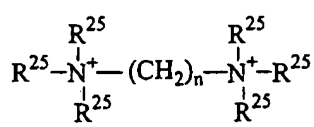


wherein $R^{12}$ is H, $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkoxy $C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, or $C_6$-$C_{18}$ aryl, $R^{13}$ is H, $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkyloxy, $C_1$-$C_6$ alkyloxy $C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, carbo($C_1$-$C_6$ alkyloxy), carbo($C_6$-$C_{18}$ aryl $C_1$-$C_6$ alkyloxy), carbo($C_6$-$C_{18}$ aryloxy), or $C_6$-$C_{18}$ aryl, and $R^{11}$ is H, OH, or $C_1$-$C_6$ alkyloxy, or $R^{11}$ and $R^{12}$ together represent



wherein each of $R^{14}$, $R^{15}$, and $R^{16}$ is, independently, H, $C_1$-$C_6$ alkyl, halogen, or trifluoromethyl, each of $R^{17}$, $R^{18}$, $R^{19}$, and $R^{20}$ are, independently, H or $C_1$-$C_6$ alkyl, and $R^{21}$ is H, halogen, trifluoromethyl, $OCF_3$, $NO_2$, $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkyloxy, $C_1$-$C_6$ alkyloxy $C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, or $C_6$-$C_{18}$ aryl.

In a second aspect, the invention features a method for treating a patient having a fungal infection caused by *Candida albicans* by administering to the patient a first compound having formula (I), above, and a second compound having the formula (III):



wherein each $R^{25}$ is, independently, $NH_2$, H, OH, a halide, $C_{1-10}$ alkyl, $C_{1-10}$ alkoxyalkyl, hydroxyalkyl (wherein the alkyl group has from 1 to 10 carbon atoms), aminoalkyl (wherein the alkyl group has from 1 to 10 carbon atoms), $C_{1-10}$ alkylaminoalkyl, cycloalkyl (wherein the alkyl group has from 1 to 10

carbon atoms), $C_{6-18}$ aryl, or $C_{1-10}$ alkylaryl; n is an integer between 2 and 10, inclusive. The first and second compounds are administered within 10 days of each other, within five days of each other, within 24 hours of each other, within one hour of each other, or are administered simultaneously of each other, in amounts sufficient to treat or inhibit the development of a fungal infection in the patient.

Desirably, the compound of formula (I) is pentamidine, propamidine, butamidine, heptamidine, nonamidine, stilbamidine, hydroxystilbamidine, diminazene, dibrompropamidine, 2,5-bis(4-amidinophenyl)furan, 2,5-bis(4-amidinophenyl)furan-bis-O-methylamidoxime, 2,5-bis(4-amidinophenyl)furan-bis-O-4-fluorophenyl, 2,5-bis(4-amidinophenyl)furan-bis-O-4-methoxyphenyl, 2,4-bis(4-amidinophenyl)furan, 2,4-bis(4-amidinophenyl)furan-bis-O-methylamidoxime, 2,4-bis(4-amidinophenyl)furan-bis-O-4-fluorophenyl, 2,4-bis(4-amidinophenyl)furan-bis-O-4-methoxyphenyl, 2,5-bis(4-amidinophenyl) thiophene, 2,5-bis(4-amidinophenyl) thiophene-bis-O-methylamidoxime, 2,4-bis(4-amidinophenyl)thiophene, 2,4-bis(4-amidinophenyl)thiophene-bis-O-methylamidoxime. Most desirably, the compound of formula (I) is pentamidine, 2,5-bis(4-amidinophenyl)furan, or 2,5-bis(4-amidinophenyl)furan-bis-O-methylamidoxime.

In a related aspect, the invention features a method for treating a patient who has a fungal infection, or inhibiting the development of a fungal infection in a patient who is at risk for developing a fungal infection. This method includes the steps of administering to the patient (i) an aromatic diamidine or a compound having formula (I); and (ii) an aminopyridine, a quaternary ammonium compound, or a compound having one of formulas (II) and (III). The two compounds are administered within 14 days of each other, within five days of each other, within 24 hours of each other, within one hour of each other, or are administered simultaneously, in amounts sufficient to treat or inhibit a fungal infection in a patient.

In a related aspect, the invention features a method for treating a patient who has a fungal infection, or inhibiting the development of a fungal infection in a patient who is at risk for developing a fungal infection. This method includes the steps of administering to the patient (i) pentamidine, propamidine, butamidine, heptamidine, nonamidine, stilbamidine, hydroxystilbamidine, diminazene, benzamidine, phenamidine, dibrompropamidine, 1,3-bis(4-amidino-2-methoxyphenoxy)propane, phenamidine, amicarbalide, 1,5-bis(4'-(N-hydroxyamidino)phenoxy)pentane, 1,3-bis(4'-(N-hydroxyamidino)phenoxy)propane, 1,3-bis(2'-methoxy-4'-(N-hydroxyamidino)phenoxy)propane, 1,4-bis(4'-(N-hydroxyamidino)phenoxy)butane, 1,5-bis(4'-(N-hydroxyamidino)phenoxy)pentane, 1,4-bis(4'-(N-hydroxyamidino)phenoxy)butane, 1,3-bis(4'-(4-hydroxyamidino)phenoxy)propane, 1,3-bis(2'-methoxy-4'-(N-hydroxyamidino)phenoxy)propane, 2,5-bis[4-amidinophenyl]furan, 2,5-bis[4-amidinophenyl]furan-bis-amidoxime, 2,5-bis[4-amidinophenyl]furan-bis-O-methylamidoxime, 2,5-bis[4-amidinophenyl]furan-bis-O-ethylamidoxime, 2,5-bis(4-amidinophenyl)furan-bis-O-4-fluorophenyl, 2,5-bis(4-amidinophenyl)furan-bis-O-4-methoxyphenyl, 2,4-bis(4-amidinophenyl)furan, 2,4-bis(4-amidinophenyl)furan-bis-O-methylamidoxime, 2,4-bis(4-amidinophenyl)furan-bis-O-4-fluorophenyl, 2,4-bis(4-amidinophenyl)furan-bis-O-4-methoxyphenyl, 2,5-bis(4-amidinophenyl) thiophene, 2,5-bis(4-amidinophenyl) thiophene-bis-O-methylamidoxime, 2,4-bis(4-amidinophenyl)thiophene, 2,4-bis(4-amidinophenyl)thiophene-bis-O-methylamidoxime, 2,8-diamidinodibenzothiophene, 2,8-bis(N-isopropylamidino)carbazole, 2,8-bis(N-hydroxyamidino)carbazole, 2,8-bis(2-imidazolinyl)dibenzothiophene, 2,8-bis(2-imidazolinyl)-5,5-dioxodibenzothiophene, 3,7-diamidinodibenzothiophene, 3,7-bis(N-isopropylamidino)dibenzothiophene, 3,7-bis(N-

hydroxyamidino)dibenzothiophene, 3,7-diaminodibenzothiophene, 3,7-dibromodibenzothiophene, 3,7-dicyanodibenzothiophene, 2,8-diamidinodibenzofuran, 2,8-di(2-imidazolinyl)dibenzofuran, 2,8-di(N-isopropylamidino)dibenzofuran, 2,8-di(N-hydroxylamidino)dibenzofuran, 3,7-di(2-imidazolinyl)dibenzofuran, 3,7-di(isopropylamidino)dibenzofuran, 3,7-di(N-hydroxylamidino)dibenzofuran, 2,8-dicyanodibenzofuran, 4,4'-dibromo-2,2'-dinitrobiphenyl, 2-methoxy-2'-nitro-4,4'-dibromobiphenyl, 2-methoxy-2'-amino-4,4'-dibromobiphenyl, 3,7-dibromodibenzofuran, 3,7-dicyanodibenzofuran, 2,5-bis(5-amidino-2-benzimidazolyl)pyrrole, 2,5-bis[5-(2-imidazolinyl)-2-benzimidazolyl]pyrrole, 2,6-bis[5-(2-imidazolinyl)-2-benzimidazolyl]pyridine, 1-methyl-2,5-bis(5-amidino-2-benzimidazolyl)pyrrole, 1-methyl-2,5-bis[5-(2-imidazolyl)-2-benzimidazolyl]pyrrole, 1-methyl-2,5-bis[5-(1,4,5,6-tetrahydro-2-pyrimidinyl)-2-benzimidazolyl]pyrrole, 2,6-bis(5-amidino-2-benzimidazoyl)pyridine, 2,6-bis[5-(1,4,5,6-tetrahydro-2-pyrimidinyl)-2-benzimidazolyl]pyridine, 2,5-bis(5-amidino-2-benzimidazolyl)furan, 2,5-bis-[5-(2-imidazolinyl)-2-benzimidazolyl]furan, 2,5-bis-(5-N-isopropylamidino-2-benzimidazolyl)furan, 2,5-bis-(4-guanylphenyl)furan, 2,5-bis(4-guanylphenyl)-3,4-dimethylfuran, 2,5-bis{p-[2-(3,4,5,6-tetrahydropyrimidyl)phenyl]}furan, 2,5-bis[4-(2-imidazolinyl)phenyl]furan, 2,5[bis-{4-(2-tetrahydropyrimidinyl)}phenyl]-3-(p-tolyloxy)furan, 2,5[bis{4-(2-imidazolinyl)}phenyl]-3-(p-tolyloxy)furan, 2,5-bis{4-[5-(N-2-aminoethylamido)benzimidazol-2-yl]phenyl}furan, 2,5-bis[4-(3a,4,5,6,7,7a-hexahydro-1H-benzimidazol-2-yl)phenyl]furan, 2,5-bis[4-(4,5,6,7-tetrahydro-1H-1,3-diazepin-2-yl)phenyl]furan, 2,5-bis(4-N,N-dimethylcarboxhydrazidephenyl)furan, 2,5-bis{4-[2-(N-2-hydroxyethyl)imidazolinyl]phenyl}furan, 2,5-bis[4-(N-isopropylamidino)phenyl]furan, 2,5-bis{4-[3-(dimethylaminopropyl)amidino]phenyl}furan, 2,5-bis{4-[N-(3-aminopropyl)amidino]phenyl}furan, 2,5-bis[2-(imidzaolinyl)phenyl]-3,4-

bis(methoxymethyl)furan, 2,5-bis[4-N-(dimethylaminoethyl)guanyl]phenylfuran, 2,5-bis{4-[(N-2-hydroxyethyl)guanyl]phenyl}furan, 2,5-bis[4-N-(cyclopropylguanyl)phenyl]furan, 2,5-bis[4-(N,N-diethylaminopropyl)guanyl]phenylfuran, 2,5-bis{4-[2-(N-ethylimidazolinyl)]phenyl}furan, 2,5-bis{4-[N-(3-pentylguanyl)]}phenylfuran, 2,5-bis[4-(2-imidazolinyl)phenyl]-3-methoxyfuran, 2,5-bis[4-(N-isopropylamidino)phenyl]-3-methylfuran, bis[5-amidino-2-benzimidazolyl]methane, bis[5-(2-imidazolyl)-2-benzimidazolyl]methane, 1,2-bis[5-amidino-2-benzimidazolyl]ethane, 1,2-bis[5-(2-imidazolyl)-2-benzimidazolyl]ethane, 1,3-bis[5-amidino-2-benzimidazolyl]propane, 1,3-bis[5-(2-imidazolyl)-2-benzimidazolyl]propane, 1,4-bis[5-amidino-2-benzimidazolyl]propane, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]butane, 1,8-bis[5-amidino-2-benzimidazolyl]octane, trans-1,2-bis[5-amidino-2-benzimidazolyl]ethene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-1-butene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-2-butene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-1-methylbutane, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-2-ethylbutane, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-1-methyl-1-butene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-2,3-diethyl-2-butene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-1,3-butadiene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-2-methyl-1,3-butadiene, bis[5-(2-pyrimidyl)-2-benzimidazolyl]methane, 1,2-bis[5-(2-pyrimidyl)-2-benzimidazolyl]ethane, 1,3-bis[5-amidino-2-benzimidazolyl]propane, 1,3-bis[5-(2-pyrimidyl)-2-benzimidazolyl]propane, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]butane, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-1-butene, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-2-butene, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-1-methylbutane, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-2-ethylbutane, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-1-methyl-1-butene, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-2,3-diethyl-2-butene, 1,4-bis[5-(2-pyrimidyl)-2-

benzimidazolyl]-1,3-butadiene, and 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-2-methyl-1,3-butadiene, 2,4-bis(4-guanylphenyl)pyrimidine, 2,4-bis(4-imidazolin-2-yl)pyrimidine, 2,4-bis[(tetrahydropyrimidinyl-2-yl)phenyl]pyrimidine, 2-(4-[N-i-propylguanyl]phenyl)-4-(2-methoxy-4-[N-i-propylguanyl]phenyl)pyrimidine, 4-(N-cyclopentylamidino)-1,2-phenylene diamine, 2,5-bis-[2-(5-amidino)benzimidazoyl]furan, 2,5-bis[2-{5-(2-imidazolino)}benzimidazoyl]furan, 2,5-bis[2-(5-N-isopropylamidino)benzimidazoyl]furan, 2,5-bis[2-(5-N-cyclopentylamidino)benzimidazoyl]furan, 2,5-bis[2-(5-amidino)benzimidazoyl]pyrrole, 2,5-bis[2-{5-(2-imidazolino)}benzimidazoyl]pyrrole, 2,5-bis[2-(5-N-isopropylamidino)benzimidazoyl]pyrrole, 2,5-bis[2-(5-N-cyclopentylamidino)benzimidazoyl]pyrrole, 1-methyl-2,5-bis[2-(5-amidino)benzimidazoyl]pyrrole, 2,5-bis[2-{5-(2-imidazolino)}benzimidazoyl]-1-methylpyrrole, 2,5-bis[2-(5-N-cyclopentylamidino)benzimidazoyl]-1-methylpyrrole, 2,5-bis[2-(5-N-isopropylamidino)benzimidazoyl]thiophene, 2,6-bis[2-{5-(2-imidazolino)}benzimidazoyl]pyridine, 2,6-bis[2-(5-amidino)benzimidazoyl]pyridine, 4,4'-bis[2-(5-N-isopropylamidino)benzimidazoyl]-1,2-diphenylethane, 4,4'-bis[2-(5-N-cyclopentylamidino)benzimidazoyl]-2,5-diphenylfuran, 2,5-bis[2-(5-amidino)benzimidazoyl]benzo[b]furan, 2,5-bis[2-(5-N-cyclopentylamidino)benzimidazoyl]benzo[b]furan, 2,7-bis[2-(5-N-isopropylamidino)benzimidazoyl]fluorine, 2,5-bis[4-(3-(N-morpholinopropyl)carbamoyl)phenyl]furan, 2,5-bis[4-(2-N,N-dimethylaminoethylcarbamoyl)phenyl]furan, 2,5-bis[4-(3-N,N-dimethylaminopropylcarbamoyl)phenyl]furan, 2,5-bis[4-(3-N-methyl-3-N-phenylaminopropylcarbamoyl)phenyl]furan, 2,5-bis[4-(3-N, $N^8$,$N^{11}$-trimethylaminopropylcarbamoyl)phenyl]furan, 2,5-bis[3-amidinophenyl]furan, 2,5-bis[3-(N-isopropylamidino)amidinophenyl]furan, 2,5-bis[3[(N-(2-

dimethylaminoethyl)amidino]phenylfuran, 2,5-bis[4-(N-2,2,2-trichloroethoxycarbonyl)amidinophenyl]furan, 2,5-bis[4-(N-thioethylcarbonyl) amidinophenyl]furan, 2,5-bis[4-(N-benzyloxycarbonyl)amidinophenyl]furan, 2,5-bis[4-(N-phenoxycarbonyl)amidinophenyl]furan, 2,5-bis[4-(N-(4-fluoro)-phenoxycarbonyl)amidinophenyl]furan, 2,5-bis[4-(N-(4-methoxy)phenoxycarbonyl)amidinophenyl]furan, 2,5-bis[4(1-acetoxyethoxycarbonyl)amidinophenyl]furan, and 2,5-bis[4-(N-(3-fluoro)phenoxycarbonyl)amidinophenyl]furan, or a salt of any of the above; and (ii) an aminopyridine, a quaternary ammonium compound, or a compound having one of formulas (II) and (III). The two compounds are administered within 14 days of each other, within five days of each other, within 24 hours of each other, within one hour of each other, or are administered simultaneously, in amounts sufficient to treat or inhibit a fungal infection in a patient.

Fungal infections treated according to the invention can cause the following conditions: tinea capitis, tinea corporis, tinea pedis, tinea barbae, tinea cruris, tinea versicolor, onychomycosis, perionychomycosis, pityriasis versicolor, tinea unguium, oral thrush, vaginal candidosis, respiratory tract candidosis, biliary candidosis, eosophageal candidosis, urinary tract candidosis, systemic candidosis, mucocutaneous candidosis, mycetoma, cryptococcosis, aspergillosis, mucormycosis, chromoblastomycosis, paracoccidioidomycosis, North American blastomycosis, histoplasmosis, coccidioidomycosis, or sporotrichosis.

In another aspect, the invention features a method of preventing, stabilizing, or inhibiting the growth of fungal cells. This method includes the steps of contacting fungal cells with (i) an aromatic diamidine or a compound having formula (I); and (ii) an aminopyridine, a quaternary ammonium compound, or a compound having one of formulas (II) and (III). The two compounds are contacted with the fungal cells in amounts that, in combination, are sufficient to prevent, stabilize, or inhibit the growth of the fungal cells.

In a related aspect, the invention features a method for preventing, stabilizing, or inhibiting the growth of fungal cells on a surface. This method includes the steps of contacting a surface with an amount of a combination of (i) an aromatic diamidine or a compound having formula (I); and (ii) an aminopyridine, a quaternary ammonium compound, or a compound having one of formulas (II) and (III), sufficient to prevent stabilize, or inhibit growth of fungal cells.

Exemplary surfaces include, for example, process equipment, water sanitation systems, cooking utensils, food preparation areas, and medical devices such as, surgical and dental tools, dental appliances including dentures, oral rinses, stents, endoscopy equipment, surgical implants, prosthetic devices, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, and continuous ambulatory peritoneal dialysis catheters.

In another aspect, the invention features a composition that includes an aromatic diamidine or a compound having formula (I), and an aminopyridine, a quaternary ammonium compound, or a compound having one of formulas (II) and (III). The two compounds are each present in amounts that, when administered together to a patient or contacted with fungal cells, inhibit or reduce fungal growth.

In a related aspect, the invention features a pharmaceutical pack that includes (i) an aromatic diamidine or a compound having formula (I); and (ii) an aminopyridine, a quaternary ammonium compound, or a compound having one of formulas (II) and (III).

In any of the foregoing aspects, the aromatic diamidine is administered or prepared for administration in an amount between 0.001 and 2000 mg per day, desirably, between 25 and 800 mg per day. The aminopyridine is administered in an amount between 0.001 and 2400 mg per day, desirably, between 10 and 1200 mg per day. The aminopyridine and aromatic diamidine

may be administered at a ratio of 2-1000 parts by molar ratio of aminopyridine to one part by molar ratio of an aromatic diamidine. The quaternary ammonium compound is administered in an amount between 0.001 and 500 mg per day, desirably, between 0.5 and 200 mg per day. The quaternary ammonium compound and aromatic diamidine may be administered, e.g., at a ratio of 2-1000 parts, by molar ratio, of quaternary ammonium compound to one part, by molar ratio, of an aromatic diamidine. Formulation and administration of the aromatic diamidine and quaternary ammonium compound can be prepared for intravenous, intramuscular, transdermal, rectal, oral, topical, intravaginal, ophthalmic, or inhalation administration. Formulation and administration of compound combination of the invention can be prepared for intravenous, intramuscular, transdermal, rectal, oral, topical, intravaginal, ophthalmic, or inhalation administration. One or both compounds may be administered at a low dosage, as defined herein, or one or both can be administered at a high dosage or a moderate dosage.

In yet another aspect, the invention features a method for identifying combinations of compounds useful for treating a patient having a fungal infection. This method includes the steps: (a) contacting fungal cells *in vitro* with (i) an aromatic diamidine, an aminopyridine, a quaternary ammonium compound, or a compound having one of formulas (I)-(III), and (ii); a candidate compound; and (b) determining whether the combination of known compound and the candidate compound reduces growth of fungal cells relative to fungal cells contacted with the known compound but not contacted with the candidate compound, or fungal cells contacted with the candidate compound but not with the known compound. A decrease in fungal growth identifies a combination as a combination that is useful for treating a patient having a fungal infection.

The invention also features a method for treating a patient who has a fungal infection, or inhibiting the development of a fungal infection in a patient who is at risk for developing a fungal infection. This method includes the steps of using an aromatic diamidine, an aminopyridine, a quaternary ammonium compound, or a compound having one of formulas (I)-(III) as an enhancer in conjunction with an antifungal agent. Inclusion of one of these enhancers allows for less antifungal agents to be administered, or improves the therapeutic efficacy of antiproliferative activity.

Desirably, the antifungal agent is chosen from the following antifungal compounds: fluconazole, amphotericin B, nystatin, pimaricin, ketoconazole, miconazole, thiabendazole, emlkonazole, itraconazole, ravuconazole, posaconazole, voriconazole, dapsone, griseofulvin, carbol-fuchsin, clotrimazole, econazole, haloprogin, mafenide, naftifine, oxiconazole, silver sulfadiazine, sulconazole, terbinafine, amorolfine, tioconazole, tolnaftate, undecylenic acid, butoconazle, gentian violet, terconazole, flucytosine, ciclopirox, caspofungin acetate, micafungin, and V-echinocandin (LY303366).

It may also be desirable to include an inhibitor of aromatic diamidine or aminopyridine metabolism in combination with an aromatic diamidine or aminopyridine to treat fungal infections. For example, SKF-525A can inhibit the hydroxylation of aminopyridines. Inhibitors to the cytochrome P-450 monooxygenase systems would also serve to increase aminopyridine bioavailability.

Aromatic diamidines suitable for use in the invention include pentamidine, propamidine, butamidine, heptamidine, nonamidine, stilbamidine, hydroxystilbamidine, diminazene, benzamidine, 4,4'- (pentamethylenedioxy) di-, dihydrochloride, phenamidine, dibrompropamidine, 1,3-bis(4-amidino-2-methoxyphenoxy)propane, netropsin, distamycin, and phenamidine. Aminopyridines suitable for use in the invention include phenazopyridine, 4-amino-pyridine, 3,4-diaminopyridine, 2,5-diamino-4-methylpyridine, 2,3,6-

triaminopyridine, 2,4,6-triaminopyridine, and 2,6-diaminopyridine. Quaternary ammonium compounds suitable for use in the invention include pentolinium, hexamethonium, pentamethonium, tetramethylammonium, tetraethylammonium, trimethaphan, and chlorisondamine.

In any of the foregoing aspects, treatment with the aforementioned antifungal compositions is directed to one or more of the following: *Absidia corymbifera*, *Acremonium falciforme*, *A. kiliense*, *A. recifei*, *Ajellomyces dermatitidis*, *A. capsulata*, *Aspergillus spp.*, (*e.g.*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus*), *Candida spp.* (*e.g.*, *C. albicans*, *C. glabrata*, *C. guillermondii*, *C. krusei*, *C. parapsilosis*, *C. kefyr*, *C. tropicalis*), *Cryptococcus neoformans*, *Cunninghamella elegans*, *Emmonsia parva*, *Epidermophyton floccosum*, *Exophialia dermitidis*, *E. werneckii*, *E. jeanselmei*, *E. spinifera*, *E. richardsiae*, *Filobasidiella neoformans*, *Fonsecaea compacta*, *F. pedrosoi*, *Histoplasma capsulatum*, *Leptoshaeria senegarlensis*, *Madurella mycetomatis*, *M. grisea*, *Malassezia furfur*, *Microsporum spp*, *Neotestudina rosatii*, *Paracoccidioides brasiliensis*, *Penicillium marneffei*, *Phialophora verrucosa*, *Piedraia hortae*, *Pneumocystis carinii*, *Pseudallescheria boydii*, *Pyrenochaeta romeroi*, *Rhizomucor pusillus*, *Sporothrix schenckii*, *Trichophyton spp*, *Trichosporon beigelii*, *Wangiella dermatitidis* and *Xylohypha bantiana*.

By "quaternary ammonium compound" is meant any quaternary ammonium-containing compound in which the nitrogen atom has four group substituents. Quaternary ammonium compounds may be mono-, symmetrical quaternary , or asymmetrical quaternary compounds.

By "aminopyridine" is meant any pyridine ring-containing compound in which the pyridine has one, two, or three amino group substituents. Other substituents may optionally be present.

By "fungal infection" is meant the invasion of a host animal by fungal cells. For example, the infection may include the excessive growth of fungi that are normally present in or on the animal, or growth of fungi that are not

normally present in or on the animal. More generally, a fungal infection can be any situation in which the presence of a fungal population is detrimental or damaging to a host animal. Thus, an animal is "suffering" from a fungal infection when an excessive amount of a fungal population is present in or on the animal, or when the presence of a fungal population is damaging the cells or tissue of the animal. In one embodiment, the number of a particular genus or species of fungus is at least 2, 4, 6, or 8 times the number normally found in the plant or animal.

By "patient" is meant any animal (e.g., a human).

By "an effective amount" is meant the amount of a compound, in a combination of the invention, required to treat or prevent a fungal infection. The effective amount of active compound(s) used to practice the present invention for therapeutic or prophylactic treatment of conditions caused by or contributed to by a fungal infection varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.

By a "low dosage" is meant at least 10% less than the lowest standard recommended dosage of an aromatic diamidine, aminopyridine, or quaternary ammonium compound.

By a "high dosage" is meant at least 5% more than the highest standard dosage of an aromatic diamidine, aminopyridine, or quaternary ammonium compound.

By a "moderate dosage" is meant the dosage between the low dosage and the high dosage.

The combination of compounds for the treatment of fungal infections allows for the administration of lower doses of each compound and less total active compound, thus providing similar or improved efficacy with low toxicity.

Compounds of the invention (e.g., an aromatic diamidine, an aminopyridine, a quaternary ammonium compound, or a compound having one of formulas (I)-(III)) can, in combination, or separately, enhance the properties of a third agent. Inclusion of one or more of such compounds with an anti-fungal agent would allow for the administration of lower doses of each compound, providing similar or enhanced therapeutic efficacy with less associated toxicity.

As used herein, the terms "alkyl" and the prefix "alk-" are inclusive of both straight chain and branched chain saturated or unsaturated groups, and of cyclic groups, i.e., cycloalkyl and cycloalkenyl groups. Cyclic groups can be monocyclic or polycyclic and preferably have from 3 to 6 ring carbon atoms, inclusive. Exemplary cyclic groups include cyclopropyl, cyclopentyl, cyclohexyl, and adamantyl groups.

By "carbo($C_1$-$C_6$ alkoxy)" is meant an ester fragment of the structure $CO_2R$, wherein R is an alkyl group.

By "carbo($C_6$-$C_{18}$ aryl-$C_1$-$C_6$ alkoxy)" is meant an ester fragment of the structure $CO_2R$, wherein R is an alkaryl group.

By "aryl" is meant a $C_6$-$C_{18}$ carbocyclic aromatic ring or ring system. Examples of aryl groups include phenyl, naphthyl, biphenyl, fluorenyl, and indenyl groups. The term "heteroaryl" means a $C_1$- $C_9$ aromatic ring or ring systems that contains at least one ring heteroatom (e.g., O, S, N). Heteroaryl groups include furyl, thienyl, pyridyl, quinolinyl, tetrazolyl, and imidazolyl groups.

By "halide" or "halogen" is meant bromine, chlorine, iodine, or fluorine.

By "heterocycle" is meant a $C_1$- $C_9$ non-aromatic ring or ring system that contains at least one ring heteroatom (e.g., O, S, N). Heterocycles include, for example, pyrrolidinyl, tetrahydrofuranyl, morpholinyl, thiazolidinyl, and imidazolidinyl groups.

Aryl, hetero, and heterocycle groups may be unsubstituted or substituted by one or more substituents selected from the group consisting of $C_{1-6}$ alkyl, hydroxy, halo, nitro, $C_{1-6}$ alkoxy, $C_{1-6}$ alkylthio, trihalomethyl, $C_{1-6}$ acyl, carbonyl, heteroarylcarbonyl, nitrile, $C_{1-6}$ alkoxycarbonyl, oxo, alkyl (wherein the alkyl group has from 1 to 6 carbon atoms) and heteroarylalkyl (wherein the alkyl group has from 1 to 6 carbon atoms).

Compounds useful in the invention include those described herein in any of their pharmaceutically acceptable forms, including isomers such as diastereomers and enantiomers, salts, solvates, and polymorphs, thereof, as well as racemic mixtures of the compounds described herein.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

## Brief Description of Drawings

Fig. 1 is a bar graph demonstrating the antiproliferative effects of the pentamidine and phenazopyridine combination in susceptible *Candida albicans*. Suspension cultures of susceptible *C. albicans* were grown at a starting density of 500 colony forming units per mL in 10 mL RPMI supplemented with 2% glucose. Cultures were treated with pentamidine at a concentration of 0.5 μM, phenazopyridine at 5 μM, Amphotericin B at 2 μM, or a combination of pentamidine and phenazopyridine at concentrations of 0.5 μM and 5 μM, respectively. As a control, a suspension culture of susceptible *C. albicans* was left untreated.

Figs. 2A-D are photomicrographs demonstrating the antiproliferative effects of the pentamidine and phenazopyridine combination on *Aspergillus fumigatus*. At 2 μM pentamidine (Fig. 2B) no antiproliferative effect was observed relative to the control (Fig. 2D). Similarly, only a moderate antiproliferative effect was observed when cells were treated with 40 μM phenazopyridine (Fig. 2C). However, the combination had a significant antiproliferative effect in *Aspergillus fumigatus* (Fig. 2A).

Fig. 3 is a bar graph demonstrating the antiproliferative effects of the pentamidine and pentolinium combination in *C. albicans*. Suspension cultures of *C. albicans* were grown at a starting density of 500 colony forming units per mL in 10 mL RPMI supplemented with 2% glucose. Cultures were treated with pentamidine at a concentration of 2.6 μM, pentolinium at 3 μM, amphotericin B at 2 μM, or a combination of pentamidine and pentolinium at concentrations of 2.6 μM and 3 μM, respectively. As a control, a suspension culture of *C. albicans* was left untreated. Cultures were incubated for 24 hours at 32°C with shaking at 250 rpm. Following this 24-hour incubation, the absorbance of each culture was measured. Cultures were centrifuged at 3000 rpm for five minutes, the supernatant was removed, and pellets were resuspended in fresh media. One thousand particles from each culture were plated on sabouraud agar plates with no supplementary compounds and incubated overnight at 32°C. Following the overnight incubation, the actual number of colonies growing on each plate was counted.

Fig. 4 is a bar graph demonstrating the antiproliferative effects of the pentamidine and pentolinium combination in *C. albicans*. Suspension cultures of *C. albicans* were grown at a starting density of 500 colony forming units per mL in 10 mL RPMI supplemented with 2% glucose. Cultures were treated with pentamidine at a concentration of 0.5 μM, pentolinium at 3 μM, amphotericin B at 2 μM, or a combination of pentamidine and pentolinium at concentrations of 0.5 μM and 3 μM, respectively. As a control, a suspension

culture of *C. albicans* was left untreated. Cultures were incubated for 24 hours at 32°C with shaking at 250 rpm. Following this 24-hour incubation, the absorbance of each culture was measured. Cultures were centrifuged at 3000 rpm for five minutes, the supernatant was removed, and pellets were resuspended in fresh media. One thousand particles from each culture were plated on sabouraud agar plates with no supplementary compounds and incubated overnight at 32°C. Following the overnight incubation, the actual number of colonies growing on each plate was counted.

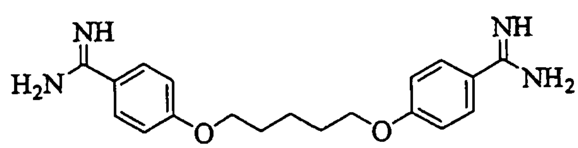
Fig. 5 is a bar graph demonstrating the antiproliferative effects of the pentamidine and pentolinium combination in *C. albicans*. Suspension cultures of *C. albicans* were grown at a starting density of 500 colony forming units per mL in 10 mL RPMI supplemented with 2% glucose. Cultures were treated with pentamidine at a concentration of 0.2 μM, pentolinium at 3 μM, amphotericin B at 2 μM, or a combination of pentamidine and pentolinium at concentrations of 0.2 μM and 3 μM, respectively. As a control, a suspension culture of *C. albicans* was left untreated. Cultures were incubated for 24 hours at 32°C with shaking at 250 rpm. Following this 24-hour incubation, the absorbance of each culture was measured. Cultures were centrifuged at 3000 rpm for five minutes, the supernatant was removed, and pellets were resuspended in fresh media. One thousand particles from each culture were plated on sabouraud agar plates with no supplementary compounds and incubated overnight at 32°C. Following the overnight incubation, the actual number of colonies growing on each plate was counted.

## Detailed Description

We have discovered that the combination of an antiprotozoal drug, pentamidine, and a diaminopyridine, phenazopyridine, or a quaternary ammonium compound, pentolinium brings about substantial inhibition of growth of triazole-resistant and triazole-susceptible strains of *C. albicans in vitro*. Concentrations that substantially inhibited fungal growth were not unacceptably toxic to normal cells. Thus, these combinations are useful for the treatment of fungal infections.

**Pentamidine**

Pentamidine is currently used for the treatment of *Pneumocystis carinii*, *Leishmania donovani*, *Trypanosoma brucei*, *T. gambiense*, and *T. rhodesiense* infections. The structure of pentamidine is:



It is available formulated for injection or inhalation. For injection, pentamidine is packaged as a nonpyrogenic, lyophilized product. After reconstitution, it is administered by intramuscular or intravenous injection.

Pentamidine isethionate is a white, crystalline powder soluble in water and glycerin and insoluble in ether, acetone, and chloroform. It is chemically designated 4,4'- diamidino-diphenoxypentane di(β-hydroxyethanesulfonate). The molecular formula is $C_{23}H_{36}N_4O_{10}S_2$ and the molecular weight is 592.68.

The mode of action of pentamidine is not fully understood. *In vitro* studies with mammalian tissues and the protozoan *Crithidia oncopelti* indicate that the drug interferes with nuclear metabolism, producing inhibition of the synthesis of DNA, RNA, phospholipids, and proteins. Several lines of

evidence suggest that the action of pentamidine against leishmaniasis, a tropical disease caused by a protozoan residing in host macrophages, might be mediated via host cellular targets and the host immune system. Pentamidine selectively targets intracellular leishmania in macrophages but not the free-living form of the protozoan and has reduced anti-leishmania activity in immunodeficient mice in comparison with its action in immunocompetent hosts.

Recently, pentamidine was shown to be an effective inhibitor of protein tyrosine phosphatase 1B (PTP1B). Because PTP1B dephosphorylates and inactivates Jak kinases, which mediate signaling of cytokines with leishmanicidal activity, its inhibition by pentamidine might result in augmentation of cytokine signaling and anti-leishmania effects. Pentamidine has also been shown to be a potent inhibitor of the oncogenic phosphatases of regenerating liver (PRL). Pentamidine has also been shown to inhibit the activity of endo-exonuclease (PCT Publication No. WO 01/35935). Thus, in the methods of the invention, pentamidine can be replaced by any PTP1B inhibitor, PRL inhibitor, or endo-exonuclease inhibitor.

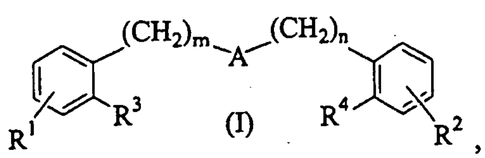
Little is known about the drug's pharmacokinetics. In seven patients treated with daily intramuscular doses of pentamidine at 4 mg/kg for 10 to 12 days, plasma concentrations were between 0.3 and 0.5 μg/mL. The patients continued to excrete decreasing amounts of pentamidine in urine up to six to eight weeks after cessation of the treatment.

Tissue distribution of pentamidine has been studied in mice given a single intraperitoneal injection of pentamidine at 10 mg/kg. The concentration in the kidneys was the highest, followed by that in the liver. In mice, pentamidine was excreted unchanged, primarily via the kidneys with some elimination in the feces. The ratio of amounts excreted in the urine and feces (4:1) was constant over the period of study.

**Pentamidine analogs**

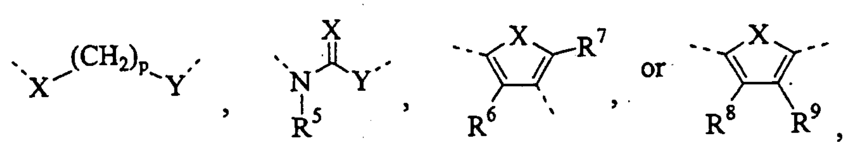
Aromatic diamidino compounds can replace pentamidine in the antifungal combination of the invention. Aromatic diamidino compounds such as propamidine, butamidine, heptamidine, and nonamidine share properties with pentamidine in that they exhibit antipathogenic or DNA binding properties. Other analogs (e.g., stilbamidine and indole analogs of stilbamidine, hydroxystilbamidine, diminazene, benzamidine, 4,4'-(pentamethylenedioxy)phenamidine, dibrompropamidine, 1,3-bis(4-amidino-2-methoxyphenoxy)propane (DAMP), netropsin, distamycin, phenamidine, amicarbalide, bleomycin, actinomycin, and daunorubicin) also exhibit properties similar to those of pentamidine. It is likely that these compounds will have antifungal activity when administered in a combination of the invention.

Pentamidine analogs are described, for example, by formula (I)

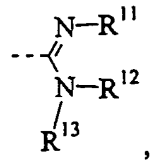


or a pharmaceutically acceptable salt thereof,

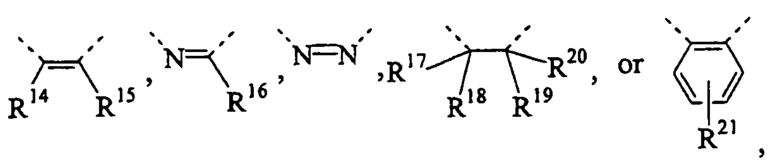
wherein A is



wherein each of X and Y is, independently, O, $NR^{10}$, or S, each of $R^5$ and $R^{10}$ is, independently, H or $C_1$-$C_6$ alkyl, each of $R^6$, $R^7$, $R^8$, and $R^9$ is, independently, H, $C_1$-$C_6$ alkyl, halogen, $C_1$-$C_6$ alkyloxy,$C_6$-$C_{18}$ aryloxy, or $C_6$-$C_{18}$ aryl-$C_1$-$C_6$ alkyloxy, p is an integer between 2 and 6, inclusive, each of m and n is, independently, an integer between 0 and 2, inclusive, each of $R^1$ and $R^2$ is

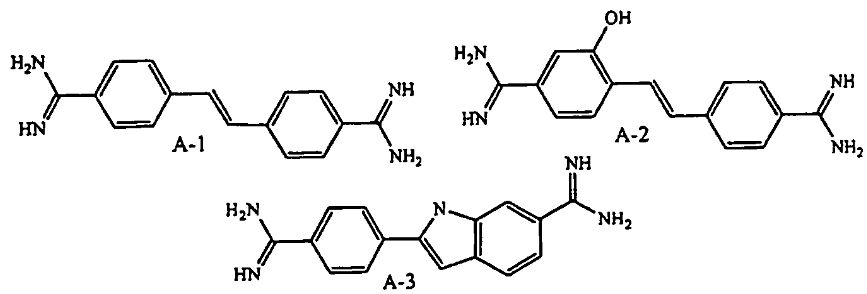


wherein $R^{12}$ is H, $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkyloxy-$C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, or $C_6$-$C_{18}$ aryl, $R^{13}$ is H, $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkyloxy, $C_1$-$C_6$ alkyloxy $C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, carbo($C_1$-$C_6$ alkyloxy), carbo($C_6$-$C_{18}$ aryl $C_1$-$C_6$ alkyloxy), carbo($C_6$-$C_{18}$ aryloxy), or $C_6$-$C_{18}$ aryl, and $R^{11}$ is H, OH, or $C_1$-$C_6$ alkyloxy, or $R^{11}$ and $R^{12}$ together represent

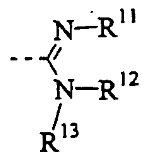


wherein each of $R^{14}$, $R^{15}$, and $R^{16}$ is, independently, H, $C_1$-$C_6$ alkyl, halogen, or trifluoromethyl, each of $R^{17}$, $R^{18}$, $R^{19}$, and $R^{20}$ is, independently, H or $C_1$-$C_6$ alkyl, and $R^{21}$ is H, halogen, trifluoromethyl, $OCF_3$, $NO_2$, $C_1$-$C_6$ alkyl, $C_1$-$C_8$ cycloalkyl, $C_1$-$C_6$ alkyloxy, $C_1$-$C_6$ alkoxy $C_1$-$C_6$ alkyl, hydroxy $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkylamino $C_1$-$C_6$ alkyl, amino $C_1$-$C_6$ alkyl, or $C_6$-$C_{18}$ aryl, each of $R^3$ and $R^4$ is, independently, H, Cl, Br, OH, $OCH_3$, $OCF_3$, $NO_2$, and $NH_2$, or $R^3$ and $R^4$ together form a single bond.

Other analogs include stilbamidine (A-1) and hydroxystilbamidine (A-2), and their indole analogs (e.g., A-3).




Each amidine moiety in A-1, A-2, or A-3 may be replaced with one of the moieties depicted in formula (I) above as


.

As is the case for pentamidine, salts of stilbamidine and its related compounds are also useful in the method of the invention. Preferred salts include, for example, dihydrochloride and methanesulfonate salts.

Still other analogs are those that fall within a formula provided in any of U.S. Patent Nos. 5,428,051; 5,521,189; 5,602,172; 5,643,935; 5,723,495; 5,843,980; 6,008,247; 6,025,398; 6,172,104; 6,214,883; and 6,326,395, or U.S. Patent Application Publication Nos. US 2001/0044468 A1 and US 2002/0019437 A1, each of which is in its entirety incorporated by reference.

Exemplary analogs are 1,3-bis(4-amidino-2-methoxyphenoxy)propane, phenamidine, amicarbalide, 1,5-bis(4'-(N-hydroxyamidino)phenoxy)pentane, 1,3-bis(4'-(N-hydroxyamidino)phenoxy)propane, 1,3-bis(2'-methoxy-4'-(N-hydroxyamidino)phenoxy)propane, 1,4-bis(4'-(N-hydroxyamidino)phenoxy)butane, 1,5-bis(4'-(N-hydroxyamidino)phenoxy)pentane, 1,4-bis(4'-(N-

hydroxyamidino)phenoxy)butane, 1,3-bis(4'-(4-hydroxyamidino)phenoxy)propane, 1,3-bis(2'-methoxy-4'-(N-hydroxyamidino)phenoxy)propane, 2,5-bis[4-amidinophenyl]furan, 2,5-bis[4-amidinophenyl]furan-bis-amidoxime, 2,5-bis[4-amidinophenyl]furan-bis-O-methylamidoxime, 2,5-bis[4-amidinophenyl]furan-bis-O-ethylamidoxime, 2,5-bis(4-amidinophenyl)furan-bis-O-4-fluorophenyl, 2,5-bis(4-amidinophenyl)furan-bis-O-4-methoxyphenyl, 2,4-bis(4-amidinophenyl)furan, 2,4-bis(4-amidinophenyl)furan-bis-O-methylamidoxime, 2,4-bis(4-amidinophenyl)furan-bis-O-4-fluorophenyl, 2,4-bis(4-amidinophenyl)furan-bis-O-4-methoxyphenyl, 2,5-bis(4-amidinophenyl) thiophene, 2,5-bis(4-amidinophenyl) thiophene-bis-O-methylamidoxime, 2,4-bis(4-amidinophenyl)thiophene, 2,4-bis(4-amidinophenyl)thiophene-bis-O-methylamidoxime, 2,8-diamidinodibenzothiophene, 2,8-bis(N-isopropylamidino)carbazole, 2,8-bis(N-hydroxyamidino)carbazole, 2,8-bis(2-imidazolinyl)dibenzothiophene, 2,8-bis(2-imidazolinyl)-5,5-dioxodibenzothiophene, 3,7-diamidinodibenzothiophene, 3,7-bis(N-isopropylamidino)dibenzothiophene, 3,7-bis(N-hydroxyamidino)dibenzothiophene, 3,7-diaminodibenzothiophene, 3,7-dibromodibenzothiophene, 3,7-dicyanodibenzothiophene, 2,8-diamidinodibenzofuran, 2,8-di(2-imidazolinyl)dibenzofuran, 2,8-di(N-isopropylamidino)dibenzofuran, 2,8-di(N-hydroxylamidino)dibenzofuran, 3,7-di(2-imidazolinyl)dibenzofuran, 3,7-di(isopropylamidino)dibenzofuran, 3,7-di(N-hydroxylamidino)dibenzofuran, 2,8-dicyanodibenzofuran, 4,4'-dibromo-2,2'-dinitrobiphenyl, 2-methoxy-2'-nitro-4,4'-dibromobiphenyl, 2-methoxy-2'-amino-4,4'-dibromobiphenyl, 3,7-dibromodibenzofuran, 3,7-dicyanodibenzofuran, 2,5-bis(5-amidino-2-benzimidazolyl)pyrrole, 2,5-bis[5-(2-imidazolinyl)-2-benzimidazolyl]pyrrole, 2,6-bis[5-(2-imidazolinyl)-2-benzimidazolyl]pyridine, 1-methyl-2,5-bis(5-amidino-2-benzimidazolyl)pyrrole, 1-methyl-2,5-bis[5-(2-imidazolyl)-2-

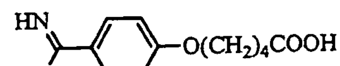
benzimidazolyl]pyrrole, 1-methyl-2,5-bis[5-(1,4,5,6-tetrahydro-2-pyrimidinyl)-2-benzimidazolyl]pyrrole, 2,6-bis(5-amidino-2-benzimidazoyl)pyridine, 2,6-bis[5-(1,4,5,6-tetrahydro-2-pyrimidinyl)-2-benzimidazolyl]pyridine, 2,5-bis(5-amidino-2-benzimidazolyl)furan, 2,5-bis-[5-(2-imidazolinyl)-2-benzimidazolyl]furan, 2,5-bis-(5-N-isopropylamidino-2-benzimidazolyl)furan, 2,5-bis-(4-guanylphenyl)furan, 2,5-bis(4-guanylphenyl)-3,4-dimethylfuran, 2,5-bis{p-[2-(3,4,5,6-tetrahydropyrimidyl)phenyl]}furan, 2,5-bis[4-(2-imidazolinyl)phenyl]furan, 2,5[bis-{4-(2-tetrahydropyrimidinyl)}phenyl]-3-(p-tolyloxy)furan, 2,5[bis{4-(2-imidazolinyl)}phenyl]-3-(p-tolyloxy)furan, 2,5-bis{4-[5-(N-2-aminoethylamido)benzimidazol-2-yl]phenyl}furan, 2,5-bis[4-(3a,4,5,6,7,7a-hexahydro-1H-benzimidazol-2-yl)phenyl]furan, 2,5-bis[4-(4,5,6,7-tetrahydro-1H-1,3-diazepin-2-yl)phenyl]furan, 2,5-bis(4-N,N-dimethylcarboxhydrazidephenyl)furan, 2,5-bis{4-[2-(N-2-hydroxyethyl)imidazolinyl]phenyl}furan, 2,5-bis[4-(N-isopropylamidino)phenyl]furan, 2,5-bis{4-[3-(dimethylaminopropyl)amidino]phenyl}furan, 2,5-bis{4-[N-(3-aminopropyl)amidino]phenyl}furan, 2,5-bis[2-(imidzaolinyl)phenyl]-3,4-bis(methoxymethyl)furan, 2,5-bis[4-N-(dimethylaminoethyl)guanyl]phenylfuran, 2,5-bis{4-[(N-2-hydroxyethyl)guanyl]phenyl}furan, 2,5-bis[4-N-(cyclopropylguanyl)phenyl]furan, 2,5-bis[4-(N,N-diethylaminopropyl)guanyl]phenylfuran, 2,5-bis{4-[2-(N-ethylimidazolinyl)]phenyl}furan, 2,5-bis{4-[N-(3-pentylguanyl)]}phenylfuran, 2,5-bis[4-(2-imidazolinyl)phenyl]-3-methoxyfuran, 2,5-bis[4-(N-isopropylamidino)phenyl]-3-methylfuran, bis[5-amidino-2-benzimidazolyl]methane, bis[5-(2-imidazolyl)-2-benzimidazolyl]methane, 1,2-bis[5-amidino-2-benzimidazolyl]ethane, 1,2-bis[5-(2-imidazolyl)-2-benzimidazolyl]ethane, 1,3-bis[5-amidino-2-benzimidazolyl]propane, 1,3-bis[5-(2-imidazolyl)-2-benzimidazolyl]propane, 1,4-bis[5-amidino-2-

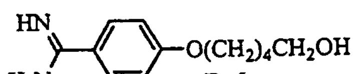
benzimidazolyl]propane, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]butane, 1,8-bis[5-amidino-2-benzimidazolyl]octane, trans-1,2-bis[5-amidino-2-benzimidazolyl]ethene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-1-butene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-2-butene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-1-methylbutane, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-2-ethylbutane, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-1-methyl-1-butene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-2,3-diethyl-2-butene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-1,3-butadiene, 1,4-bis[5-(2-imidazolyl)-2-benzimidazolyl]-2-methyl-1,3-butadiene, bis[5-(2-pyrimidyl)-2-benzimidazolyl]methane, 1,2-bis[5-(2-pyrimidyl)-2-benzimidazolyl]ethane, 1,3-bis[5-amidino-2-benzimidazolyl]propane, 1,3-bis[5-(2-pyrimidyl)-2-benzimidazolyl]propane, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]butane, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-1-butene, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-2-butene, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-1-methylbutane, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-2-ethylbutane, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-1-methyl-1-butene, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-2,3-diethyl-2-butene, 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-1,3-butadiene, and 1,4-bis[5-(2-pyrimidyl)-2-benzimidazolyl]-2-methyl-1,3-butadiene, 2,4-bis(4-guanylphenyl)pyrimidine, 2,4-bis(4-imidazolin-2-yl)pyrimidine, 2,4-bis[(tetrahydropyrimidinyl-2-yl)phenyl]pyrimidine, 2-(4-[N-i-propylguanyl]phenyl)-4-(2-methoxy-4-[N-i-propylguanyl]phenyl)pyrimidine, 4-(N-cyclopentylamidino)-1,2-phenylene diamine, 2,5-bis-[2-(5-amidino)benzimidazoyl]furan, 2,5-bis[2-{5-(2-imidazolino)}benzimidazoyl]furan, 2,5-bis[2-(5-N-isopropylamidino)benzimidazoyl]furan, 2,5-bis[2-(5-N-cyclopentylamidino)benzimidazoyl]furan, 2,5-bis[2-(5-amidino)benzimidazoyl]pyrrole, 2,5-bis[2-{5-(2-imidazolino)}benzimidazoyl]pyrrole, 2,5-bis[2-(5-N-isopropylamidino)benzimidazoyl]pyrrole, 2,5-bis[2-(5-N-

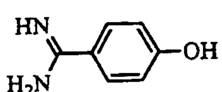
cyclopentylamidino)benzimidazoyl]pyrrole, 1-methyl-2,5-bis[2-(5-amidino)benzimidazoyl]pyrrole, 2,5-bis[2-{5-(2-imidazolino)}benzimidazoyl]-1-methylpyrrole, 2,5-bis[2-(5-N-cyclopentylamidino)benzimidazoyl]-1-methylpyrrole, 2,5-bis[2-(5-N-isopropylamidino)benzimidazoyl]thiophene, 2,6-bis[2-{5-(2-imidazolino)}benzimidazoyl]pyridine, 2,6-bis[2-(5-amidino)benzimidazoyl]pyridine, 4,4'-bis[2-(5-N-isopropylamidino)benzimidazoyl]-1,2-diphenylethane, 4,4'-bis[2-(5-N-cyclopentylamidino)benzimidazoyl]-2,5-diphenylfuran, 2,5-bis[2-(5-amidino)benzimidazoyl]benzo[b]furan, 2,5-bis[2-(5-N-cyclopentylamidino)benzimidazoyl]benzo[b]furan, 2,7-bis[2-(5-N-isopropylamidino)benzimidazoyl]fluorene, 2,5-bis[4-(3-(N-morpholinopropyl)carbamoyl)phenyl]furan, 2,5-bis[4-(2-N,N-dimethylaminoethylcarbamoyl)phenyl]furan, 2,5-bis[4-(3-N,N-dimethylaminopropylcarbamoyl)phenyl]furan, 2,5-bis[4-(3-N-methyl-3-N-phenylaminopropylcarbamoyl)phenyl]furan, 2,5-bis[4-(3-N, $N^8$,$N^{11}$-trimethylaminopropylcarbamoyl)phenyl]furan, 2,5-bis[3-amidinophenyl]furan, 2,5-bis[3-(N-isopropylamidino)amidinophenyl]furan, 2,5-bis[3[(N-(2-dimethylaminoethyl)amidino]phenylfuran, 2,5-bis[4-(N-2,2,2-trichloroethoxycarbonyl)amidinophenyl]furan, 2,5-bis[4-(N-thioethylcarbonyl) amidinophenyl]furan, 2,5-bis[4-(N-benzyloxycarbonyl)amidinophenyl]furan, 2,5-bis[4-(N-phenoxycarbonyl)amidinophenyl]furan, 2,5-bis[4-(N-(4-fluoro)-phenoxycarbonyl)amidinophenyl]furan, 2,5-bis[4-(N-(4-methoxy)phenoxycarbonyl)amidinophenyl]furan, 2,5-bis[4(1-acetoxyethoxycarbonyl)amidinophenyl]furan, and 2,5-bis[4-(N-(3-fluoro)phenoxycarbonyl)amidinophenyl]furan. Methods for making any of the foregoing compounds are described in U.S. Patent Nos. 5,428,051; 5,521,189; 5,602,172; 5,643,935; 5,723,495; 5,843,980; 6,008,247; 6,025,398; 6,172,104; 6,214,883; and 6,326,395, an U.S. Patent Application Publication Nos. US 2001/0044468 A1 and US 2002/0019437 A1.

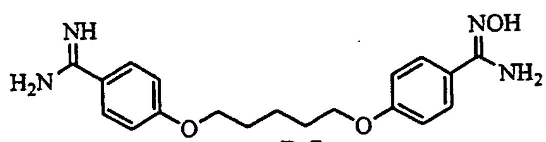
**Pentamidine Metabolites**

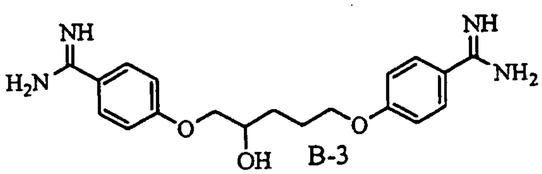
Pentamidine metabolites are also useful in the antifungal combination of the invention. Pentamidine is rapidly metabolized in the body to at least seven primary metabolites. Some of these metabolites share one or more activities with pentamidine. It is likely that some pentamidine metabolites will have antifungal activity when administered in combination with an antiproliferative agent. Seven pentamidine metabolites (B-1 through B-7) are shown below.

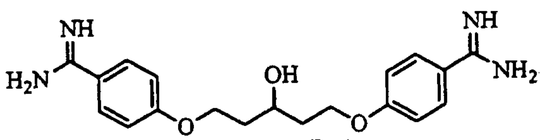

B-1

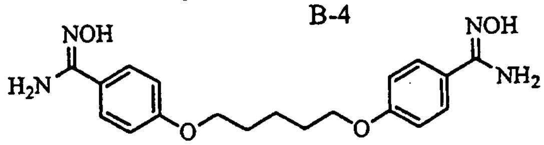

B-6


B-2


B-7

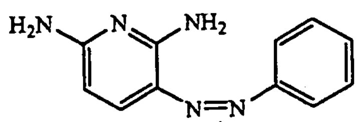

B-3


B-4

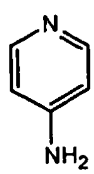

B-5

**Phenazopyridine**

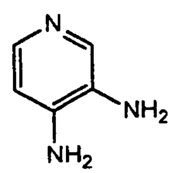
By "aminopyridine" is meant any pyridine ring-containing compound in which the pyridine has one, two, or three amino group substituents. Other substituents may optionally be present. Aminopyridines include phenazopyridine (C-1), 4-aminopyridine (C-2), 3,4-diaminopyridine (C-3), 2,5-diamino-4-methylpyridine (C-4), 2,3,6-triaminopyridine (C-5), 2,4,6-triaminopyridine (C-6), and 2,6-diaminopyridine (C-7), the structures of which are depicted below.



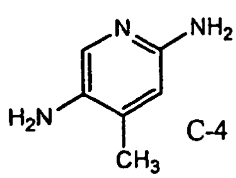

C-1




C-2




C-3

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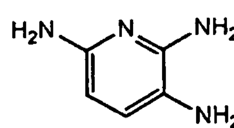
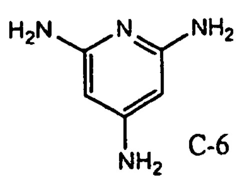
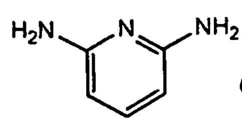



C-5




C-6

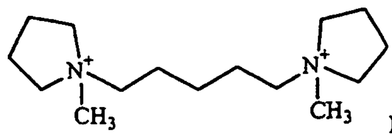



C-7

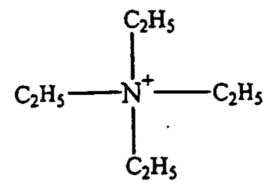
Aminopyridines can accommodate many modifications while still maintaining structural and therapeutic efficacy. Phenazopyridine and derivatives thereof have been disclosed in U.S. Patent Nos. 1,680,108, 1,680,109, 1,680,110, and 1,680,111. Heterocyclic azo derivatives and N-substituted diaminopyridines have also been described (see, e.g., U.S. Patent Nos. 2,145,579 and 3,647,808).

**Quaternary ammonium compounds**

Quaternary ammonium compounds include, for example, pentolinium (D-1), hexamethonium (D-2), pentamethonium (D-3), tetraethylammonium (D-4), tetramethylammonium (D-5), chlorisondamine (D-6), and trimethaphan (D-7), the structures of which are depicted below.



D-1



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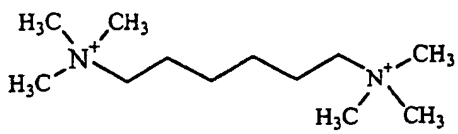

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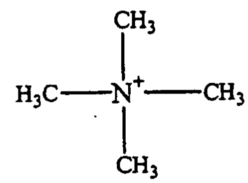

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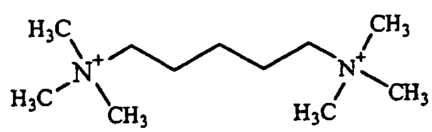
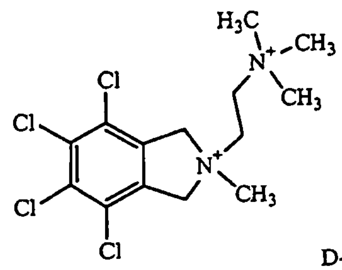
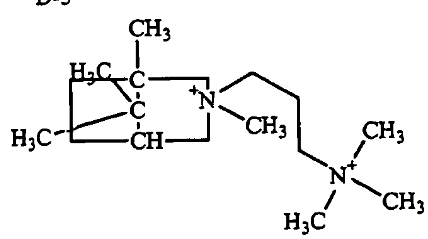


D-5



D-6



D-7

Pentolinium (pentamethylene-1,5-bis(N-methylpyrrolidinium) and its salt, pentolinium ditartrate, are symmetrical quaternary ammonium compounds. The tartrate salt form of pentolinium has the molecular formula $C_{23}H_{42}N_2O_{12}$ with a molecular weight of 538.6. Pentolinium ditartrate is a white powder, near odorless, and highly soluble in water.

*Pentolinium analogs*

Quaternary ammonium compounds can accommodate many modifications while still maintaining structural and therapeutic efficacy. Pentolinium and its derivatives thereof are described in U.S. Patent Nos. 4,902,720 and 6,096,788, each of which is herein incorporated by reference. Any of the quaternary ammonium compounds described in the foregoing patents can be used in a combination of the invention.

**Therapy**

Combination therapy according to the invention may be performed alone or in conjunction with another therapy and may be provided at home, the doctor's office, a clinic, a hospital's outpatient department, or a hospital. Treatment generally begins at a hospital so that the doctor can observe the therapy's effects closely and make any adjustments that are needed. The duration of the combination therapy depends on the age and condition of the patient, the stage of the patient's disease, and how the patient responds to the treatment. Additionally, a person having a greater risk of developing a fungal infection (e.g., a person who is to undergo a surgical procedure) may receive prophylactic treatment to inhibit or delay the onset of symptoms.

The dosage, frequency and mode of administration of each component of the combination can be controlled independently. For example, one compound may be administered orally three times per day, while the second compound may be administered topically once per day. Combination therapy may be given in on-and-off cycles that include rest periods so that the patient's body has a chance to recovery from any as yet unforeseen side-effects. The compounds may also be formulated together such that one administration delivers both compounds.

**Formulation of pharmaceutical compositions**

The administration of each compound of the combination may be by any suitable means that results in a concentration of the compound that, combined with the other component, is antifungal. The compound may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for the oral, parenteral (e.g., intravenously, intramuscularly), rectal, cutaneous, nasal, vaginal, inhalant, skin (patch), or ocular administration route. Thus, the composition may be in the form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, osmotic delivery devices, suppositories, enemas, injectables, implants, sprays, or aerosols. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: *The Science and Practice of Pharmacy*, 20th edition, 2000, ed. A.R. Gennaro, Lippincott Williams & Wilkins, Philadelphia, and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

**Dosages**

The dosage of each compound of the claimed combinations depends on several factors, including: the administration method, the disease to be treated, the severity of the disease, whether the disease is to be treated or prevented, and the age, weight, and health of the person to be treated. While suggested dosages will vary with a patient's condition, standard recommended dosages are provided below. The standard recommended dosage of pentamidine is 4 mg/kg i.v. or 300 mg oral. The standard recommended dosage of phenazopyridine is 200 mg, three times per day. For oral administration of the quaternary ammonium compounds, the standard recommended dosage is about

20 mg, taken three times daily. Pentolinium ditartrate can be administered by intramuscular or subcutaneous injection at standard recommended dosage of 3.5 mg, and increased as needed. Intravenous administration of pentolinium is recommended as a 3 mg IV bolus followed by sustained injection 0.1-0.3 mg/kg body weight over ten minutes.

As described above, the compound in question may be administered orally in the form of tablets, capsules, elixirs or syrups, or rectally in the form of suppositories. Parenteral administration of a compound is suitably performed, for example, in the form of saline solutions or with the compound incorporated into liposomes. In cases where the compound in itself is not sufficiently soluble to be dissolved, a solubilizer such as ethanol can be applied. Below, for illustrative purposes, the dosages for phenazopyridine and pentamidine are described. One in the art will recognize that if an alternative compound is substituted for either a phenazopyridine or pentamidine, the correct dosage can be determined by examining the efficacy of the compound in cell proliferation assays, as well as its toxicity in humans.

For oral administration of the quaternary ammonium compounds, the standard recommended adult dosage is about 20 mg, taken three times daily. Pentolinium ditartrate can be administered by intramuscular or subcutaneous injection at initial doses of 3.5 mg, and increased as needed. Intravenous administration of pentolinium is recommended as a 3 mg IV bolus followed by sustained injection 0.1-0.3 mg/kg body weight over ten minutes.

In a desired dose combination, the ratio of aminopyridine or quaternary ammonium compound to aromatic amidine is 1000:1, 500:1, 250:1, 100:1, 50:1, 25:1, 15:1, 10:1, 5:1, 4:1, or 2:1 by molar ratio. Enhancement is observed across the various combination ratios, however, some ratios will demonstrate better enhancement than others. All ratios are contemplated.

*Oral administration*

For an aminopyridine such as phenazopyridine, for oral administration or systemic use, the dosage is normally about 0.001 mg to 2400 mg per dose administered (desirably about 0.5 mg to 1600 mg, and more desirably about 10 mg to 1200 mg) one to ten times daily (preferably one to five times daily, more desirably one to three times daily). In a desired dose combination, the ratio of phenazopyridine to pentamidine is 1000:1, 500:1, 250:1, 100:1, 50:1, 25:1, 15:1, 10:1, 5:1, 4:1, or 2:1 by molar ratio.

For pentamidine, the dosage is normally about 0.1 mg to 300 mg per dose administered (preferably about 1 mg to 100 mg) one to four times daily for one day to one year, and, may be administered for the life of the patient. Administration may also be given in cycles, such that there are periods during which time pentamidine is not administered. This period could be, for example, about a day, a week, a month, or a year or more.

*Rectal administration*

For compositions adapted for rectal use for preventing infection, a somewhat higher amount of a compound is usually preferred. Thus a dosage of an aminopyridine is normally about 5 mg to 2400 mg per dose (preferably about 10 mg to 1200 mg) administered one to four times daily. Treatment durations are as described for oral administration. The dosage of pentamidine is as described for orally administered pentamidine.

*Parenteral administration*

For intravenous or intramuscular administration of an aminopyridine, a dose of about 0.1 mg/kg to about 100 mg/kg body weight per day is recommended, a dose of about 1 mg/kg to about 50 mg/kg is preferred, and a dose of 1 mg/kg to 25 mg/kg is most preferred. Pentamidine is administered at

a dose of about 0.1 mg/kg to about 20 mg/kg, preferably at a dose of about 0.5 mg/kg to about 10 mg/kg, and more preferably at a dose of about 1 mg/kg to about 4 mg/kg.

Each compound is usually administered daily for up to about 6 to 12 months or more. It may be desirable to administer a compound over a one to three hour period; this period may be extended to last 24 hours or more. As is described for oral administration, there may be periods of about one day to one year or longer during which at least one of the drugs is not administered.

*Inhalation*

For inhalation, an aminopyridine is administered at a dose of about 1 mg to 2400 mg daily, and preferably at a dose of about 10 mg to 1200 mg daily. For pentamidine, a dose of about 10 mg to 1000 mg, and preferably at a dose of 30 mg to 600 mg, is administered daily.

*Percutaneous administration*

For topical administration of either compound, a dose of about 1 mg to about 5 g administered one to ten times daily for one week to 12 months is usually preferable.

**Other uses**

Combinations of the invention may also be used for the preservation of food, beverages, cosmetics such as lotions, creams, gels, ointments, soaps, shampoos, conditioners, antiperspirants, deodorants, mouthwash, contact lens products, enzyme formulations, or food ingredients. Combinations of the invention can be incorporated into, for example, unpreserved food, beverages, cosmetics, contact lens products, or food ingredients in an amount effective for killing or inhibiting the growth of fungi.

Thus, a combination of the invention may be useful as a disinfectant, e.g., in the treatment of acne, eye infections, mouth infections, fingernail infections, toenail infections, skin infections, wounds, or in treating infections caused by the insertion of stents. Combinations of the invention are also useful for cleaning, disinfecting, or inhibiting fungal growth on any hard surface. Examples of surfaces which may advantageously be contacted with a combination of the invention are surfaces of process equipment used in dairies, chemical or pharmaceutical process plants, water sanitation systems, paper pulp processing plants, water treatment plants, cooling towers, cooking utensils, or surfaces in any area in which food is prepared (e.g., hospitals, nursing homes, or restaurants).

In addition, combinations of the invention are useful for cleaning, disinfecting, or inhibiting fungal growth on or in an in-dwelling device in a patient. In-dwelling devices include, but are not limited to, surgical and dental implants, prosthetic devices, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, stents, cerebrospinal fluid shunts, urinary catheters, and continuous ambulatory peritoneal dialysis (CAPD) catheters. A combination of the invention may be used to bathe an in-dwelling device immediately before insertion. Alternatively, the combination may be administered by injection to achieve a local or systemic effect against relevant fungi shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device.

The following examples are to illustrate the invention. They are not meant to limit the invention in any way.

**Example 1: pentamidine and phenazopyridine combinations for treatment of candidosis**

Using the methods described below, we tested the ability of the combination of various concentrations of pentamidine plus phenazopyridine to

inhibit the proliferation of numerous yeast strains using particle density as an indicator of cell number. In addition, we performed colony-forming assays to test the relative biocidal activity of specific concentrations of pentamidine and phenazopyridine. Finally, as a preliminary measure of the toxicity of these combinations to mammalian cells, we added the pentamidine/phenazopyridine combination to cells in culture and assayed for viable cells using the reduction of Alamar Blue.

The graph in Fig. 1 depicts the biocidal activity of the pentamidine and phenazopyridine combination in susceptible *C. albicans*. Suspension cultures of susceptible *C. albicans* were grown at a starting density of 500 colony-forming units (CFUs) per mL in 10 mL RPMI media supplemented with 2% glucose. Cultures were treated with pentamidine, phenazopyridine, amphotericin B (AmpB), or pentamidine plus phenazopyridine at the indicated concentrations. As a control, a suspension culture of susceptible *C. albicans* was left untreated. Cultures were incubated for 24 hours at 32°C with shaking at 250 rpm. Following this 24-hour incubation, the absorbance of each culture was measured. Cultures were centrifuged at 3000 rpm for five minutes, the supernatant was removed, and pellets were resuspended in fresh media. One thousand particles from each culture were plated on sabouraud agar plates with no supplementary compounds and incubated overnight at 32°C. Following the overnight incubation, the actual number of colonies growing on each plate was counted. Results indicate that treatment of susceptible *C. albicans* with either single agent suppresses growth. Removal of compounds by plating results in significant colony growth. In contrast, treatment with pentamidine and phenazopyridine not only repressed growth in suspension, *C. albicans* treated with pentamidine and phenazopyridine showed dramatically repressed colony growth following plating and after the removal of the drug combination.

Tables 1 and 2 demonstrate the antiproliferative effects of combining pentamidine-isethionate and phenazopyridine-HCl to a clinical isolate of the opportunistic yeast, *Candida albicans*. As observed in Tables 1 and 2, The $MIC_{70}$ of pentamidine for the strain 17 isolate was calculated at 0.21 µM. Combination of pentamidine with a moderate amount of phenazopyridine (5 µM) results in a greater than 75% reduction of the $MIC_{70}$ for pentamidine.

**Table 1: Percent inhibition of proliferation of *Candida albicans* strain 17**

| | Pentamidine (µM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Phenazopyridine (µM) | 3.370 | 1.685 | 0.843 | 0.421 | 0.211 | 0.105 | 0.053 | 0.026 | .013 | 0 |
| 40.00 | 96.2 | 95.9 | 96.4 | 96.5 | 96.4 | 96.4 | 89.0 | 63.7 | 44.4 | 32.8 |
| 20.00 | 95.9 | 96.2 | 96.4 | 96.4 | 96.1 | 89.7 | 57.4 | 33.7 | 22.8 | 40.2 |
| 10.00 | 95.5 | 96.0 | 96.3 | 96.3 | 95.9 | 84.2 | 35.0 | 26.0 | 4.8 | -1.4 |
| 5.00 | 95.3 | 96.2 | 96.3 | 96.2 | 65.0 | 35.3 | 8.6 | 5.8 | -0.9 | -1.1 |
| 2.50 | 93.9 | 95.5 | 96.2 | 96.1 | 78.1 | 29.4 | 15.9 | 5.7 | -7.6 | -6.4 |
| 1.25 | 94.4 | 95.8 | 96.3 | 92.7 | 52.8 | 25.0 | -1.0 | -2.2 | -3.1 | 5.4 |
| 0.63 | 92.3 | 94.6 | 96.1 | 93.8 | 53.5 | 27.2 | 4.1 | 5.7 | -0.7 | 35.1 |
| 0.31 | 92.3 | 94.9 | 96.2 | 80.5 | 41.5 | 29.0 | 16.7 | 1.1 | 6.6 | -5.5 |
| 0.16 | 87.0 | 92.4 | 95.9 | 83.5 | 50.9 | 12.4 | -0.9 | 0.2 | -0.3 | 5.9 |
| 0.00 | 88.1 | 93.8 | 95.7 | 60.0 | 30.7 | 22.2 | 9.2 | -1.0 | -5.3 | -2.6 |

**Table 2: Percent inhibition of proliferation of *Candida albicans* strain 17**

| | Phenazopyridine (µM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Pentamidine (µM) | 40.00 | 20.00 | 10.00 | 5.00 | 2.50 | 1.25 | 0.63 | 0.31 | 0.16 | 0 |
| 0.4200 | 95.9 | 94.2 | 93.0 | 89.9 | 81.3 | 78.6 | 70.2 | 74.1 | 70.9 | 71.9 |
| 0.2100 | 95.5 | 93.5 | 91.8 | 84.7 | 80.4 | 73.2 | 76.4 | 78.3 | 78.9 | 70.1 |
| 0.1050 | 97.3 | 96.4 | 93.9 | 87.4 | 87.5 | 84.2 | 84.0 | 88.3 | 83.3 | 68.6 |
| 0.0525 | 97.5 | 97.0 | 95.8 | 90.0 | 91.4 | 76.7 | 75.5 | 38.1 | 74.2 | 45.6 |
| 0.0263 | 97.6 | 97.1 | 94.3 | 79.9 | 13.0 | 16.1 | -0.4 | -4.7 | -1.6 | -4.0 |
| 0.0131 | 97.6 | 96.0 | 46.7 | 8.8 | 3.0 | -4.0 | -2.3 | -3.0 | -4.7 | -4.5 |
| 0.0066 | 96.0 | 25.5 | 3.0 | -3.0 | -4.4 | -3.9 | -4.1 | -3.5 | -3.6 | -4.9 |
| 0.0033 | 87.8 | 30.7 | 0.7 | -3.7 | -4.4 | -3.4 | -3.4 | -3.6 | -3.7 | -2.1 |
| 0.0016 | 32.6 | 9.8 | -3.9 | -2.7 | -3.5 | -4.4 | -3.9 | -3.6 | -2.7 | -3.6 |
| 0.0000 | -0.8 | -2.5 | -2.9 | -2.8 | -2.3 | -4.1 | -3.0 | -4.0 | -2.8 | -3.0 |

**Example 2: Pentamidine and phenazopyridine combinations for treatment of *Candida spp.* infections**

The effects of pentamidine and phenazopyridine were further tested on additional *Candida* species including *C. albicans*, strain MYA 573 (Table 3), a known triazole-resistant clinical isolate, *C. glabrata* (Table 4), and *I. Orientalis* (a teleomorph of *C. krusei*; Table 5).

Consistent with the previously observed antiproliferative effects of pentamidine/phenazopyridine combinatorial treatment, dose-shifting of pentamidine was demonstrated when combined with phenazopyridine. Data from the colony formation assays suggest that the concentrations of pentamidine and phenazopyridine were capable of suppressing growth up to 93% (data not shown).

**Table 3: percent inhibition of proliferation of triazole-resistant *C. albicans* strain MYA-573**

| | Phenazopyridine (µM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Pentamidine (µM) | 40.00 | 20.00 | 10.00 | 5.00 | 2.50 | 1.25 | 0.63 | 0.31 | 0.16 | 0 |
| 0.840 | 94.9 | 88.5 | 83.6 | 81.1 | 75.1 | 70.2 | 62.9 | 63.0 | 54.2 | 45.9 |
| 0.420 | 90.7 | 78.2 | 71.3 | 54.2 | 58.7 | 42.1 | 29.1 | 24.5 | 29.3 | 16.8 |
| 0.210 | 88.5 | 77.8 | 51.4 | 41.2 | 32.7 | 22.4 | 14.2 | 15.9 | 16.5 | 11.5 |
| 0.105 | 87.6 | 61.0 | 38.7 | 24.5 | 22.4 | 13.2 | 8.1 | 13.4 | 8.5 | 5.4 |
| 0.053 | 83.5 | 38.5 | 26.3 | 18.1 | 14.8 | 7.8 | 5.0 | 4.9 | 2.5 | 1.0 |
| 0.026 | 70.8 | 32.8 | 20.6 | 11.6 | 13.7 | 5.1 | 4.7 | 0.6 | 1.4 | 0.2 |
| 0.013 | 64.9 | 33.2 | 18.8 | 13.3 | 5.3 | 4.9 | 1.4 | 2.4 | 2.1 | -2.1 |
| 0.007 | 51.0 | 27.3 | 19.2 | -3.8 | 10.8 | 3.5 | 2.6 | 5.6 | -1.0 | -2.0 |
| 0.003 | 41.7 | 20.5 | 12.1 | 9.1 | 5.3 | 3.6 | 4.0 | 0.8 | 2.7 | -1.5 |
| 0.000 | 35.7 | 16.1 | 8.8 | 8.4 | -1.3 | 11.5 | -0.2 | -0.4 | -0.6 | -1.1 |

**Table 4: Percent inhibition of proliferation of *C. glabrata***

| | Phenazopyridine (µM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Pentamidine (µM) | 40.00 | 20.00 | 10.00 | 5.00 | 2.50 | 1.25 | 0.63 | 0.31 | 0.16 | 0 |
| 0.840 | 96.1 | 96.7 | 97.8 | 94.4 | 91.4 | 95.0 | 95.9 | 97.2 | 96.2 | 95.8 |
| 0.420 | 95.0 | 94.4 | 94.2 | 89.5 | 89.4 | 90.2 | 90.8 | 89.6 | 92.0 | 88.3 |
| 0.210 | 84.2 | 76.9 | 79.3 | 74.4 | 71.8 | 72.7 | 73.6 | 66.9 | 57.1 | 58.3 |
| 0.105 | 73.9 | 64.2 | 57.6 | 46.8 | 37.7 | 33.1 | 39.1 | 33.2 | 26.2 | 22.7 |
| 0.053 | 62.1 | 41.5 | 34.8 | 25.2 | 23.5 | 18.2 | 14.8 | 9.6 | 2.4 | 15.0 |
| 0.026 | 47.2 | 26.9 | 20.0 | 17.6 | 9.9 | 2.9 | -3.1 | -5.6 | -7.4 | -12 |
| 0.013 | 39.8 | 20.1 | 14.9 | 17.2 | 10.4 | 8.9 | 8.0 | -3.7 | -2.0 | -6.5 |
| 0.007 | 11.7 | 9.6 | 16.1 | 3.7 | 1.2 | 0.6 | -0.9 | -7.3 | -9.2 | -7.8 |
| 0.003 | 24.7 | 23.4 | 18.5 | 16.6 | 11.3 | 13.9 | 6.2 | 6.8 | 0.0 | -4.7 |
| 0.000 | 21.1 | 17.6 | 12.4 | 12.4 | 8.0 | 10.1 | 1.0 | -1.0 | -3.3 | -3.6 |

**Table 5: Percent inhibition of proliferation of *I. orientalis***

| | Phenazopyridine (µM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Pentamidine (µM) | 40.00 | 20.00 | 10.00 | 5.00 | 2.50 | 1.25 | 0.63 | 0.31 | 0.16 | 0 |
| 0.840 | 85.5 | 80.6 | 80.0 | 76.1 | 79.2 | 76.4 | 77.9 | 80.5 | 82.1 | 81.5 |
| 0.420 | 84.8 | 75.3 | 74.8 | 71.0 | 71.7 | 67.7 | 71.4 | 69.4 | 74.9 | 71.3 |
| 0.210 | 81.0 | 72.0 | 68.1 | 65.7 | 63.9 | 60.3 | 61.7 | 62.8 | 65.4 | 63.1 |
| 0.105 | 75.8 | 61.0 | 56.0 | 50.8 | 46.9 | 44.9 | 46.5 | 38.4 | 45.9 | 54.2 |
| 0.053 | 68.8 | 46.6 | 37.2 | 32.3 | 35.6 | 26.2 | 27.1 | 27.4 | 26.1 | 29.5 |
| 0.026 | 67.7 | 37.7 | 29.1 | 17.0 | 9.8 | 7.1 | -1.9 | 4.0 | 2.5 | 12.2 |
| 0.013 | 67.1 | 40.4 | 19.3 | 18.3 | 14.7 | 3.1 | -0.1 | 1.5 | 11.2 | 3.4 |
| 0.007 | 69.9 | 36.9 | 17.0 | 7.2 | 13.4 | 9.2 | -1.3 | 1.4 | 2.6 | -3.5 |
| 0.003 | 68.5 | 41.6 | 24.1 | 9.9 | 3.7 | -5.7 | 0.7 | -3.0 | 3.1 | 4.7 |
| 0.000 | 67.7 | 40.1 | 24.4 | 25.8 | 12.7 | 6.3 | -2.8 | 1.0 | -3.2 | 0.7 |

**Example 3: pentamidine and phenazopyridine combinations for treatment of *C. neoformans* infections**

The fungus causing cryptococcus, *C. neoformans*, is ubiquitously found in soil contaminated with pigeon or other avian excretia. *Cryptococcus* has also been found on unwashed raw fruit. Cryptococcosis is a rare disease in healthy individuals, but is one of the most common fungal infections affecting people with AIDS or patients that are immunosuppressed due to chemotherapy.

Our results show that the combination of pentamidine and phenazopyridine was able to negatively effect proliferation of the fungal cells in culture (Table 6).

**Table 6: percent inhibition of proliferation of *C. neoformans***

| Pentamidine (µM) | Phenazopyridine (µM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 40.00 | 20.00 | 10.00 | 5.00 | 2.50 | 1.25 | 0.63 | 0.31 | 0.16 | 0 |
| 0.840 | 79.7 | 72.3 | 69.6 | 69.0 | 67.7 | 69.2 | 69.1 | 67.8 | 66.7 | 62.7 |
| 0.420 | 75.7 | 66.0 | 66.4 | 61.4 | 58.9 | 57.7 | 58.9 | 55.3 | 54.0 | 53.2 |
| 0.210 | 65.7 | 55.9 | 53.5 | 49.0 | 47.9 | 52.1 | 46.4 | 48.0 | 45.6 | 39.4 |
| 0.105 | 56.7 | 41.9 | 39.0 | 37.0 | 29.6 | 32.1 | 33.9 | 33.3 | 26.3 | 29.0 |
| 0.053 | 52.9 | 32.3 | 26.7 | 31.7 | 22.3 | 27.6 | 22.9 | 21.1 | 13.2 | 15.7 |
| 0.026 | 47.5 | 23.5 | 11.4 | 7.3 | 4.6 | 7.2 | 2.1 | 1.7 | -0.2 | 2.2 |
| 0.013 | 46.2 | 22.7 | 12.4 | 9.7 | 2.8 | 3.1 | 2.3 | 0.6 | 0.2 | 1.1 |
| 0.007 | 43.6 | 21.0 | 8.1 | 2.7 | 0.0 | -0.8 | -0.7 | -2.2 | -1.7 | -2.1 |
| 0.003 | 42.1 | 18.8 | 8.4 | 5.6 | 1.8 | 2.7 | 1.8 | -0.1 | 0.5 | 1.1 |
| 0.000 | 36.3 | 14.0 | 7.8 | 2.6 | 0.0 | -1.1 | -0.6 | -2.6 | -1.5 | -0.6 |

**Example 4: pentamidine and phenazopyridine combinations for treatment of aspergillosis**

As a preliminary measure of the effect of the combination for on molds, we studied the effects of the combination of pentamidine and phenazopyridine in *A. fumigatus* cells. Figs. 2A-2D demonstrates the antiproliferative effects of combining pentamidine and phenazopyridine to a clinical isolate of *Aspergillus*. At 2 µM pentamidine (Fig. 2B) no antiproliferative effect as compared to the control (Fig. 2D). Similarly, only a moderated antiproliferative effect was observed when *Aspergillus* cells were treated with 40 µM phenazopyridine (Fig. 2C). However, as observed in the *Candida spp.*, the combination had a significant antiproliferative effect in *A. fumigatus* (Fig. 2A).

## Example 5: toxicity of pentamidine and phenazopyridine combination treatment in human cells

As a preliminary measure of toxicity, we studied the effects of the combination of pentamidine and phenazopyridine in confluent human MRC-9 fibroblast cells. MRC-9 cells are non-transformed, diploid cells that exhibit contact-inhibited cell growth characteristics. As is shown in Table 7, the combination of pentamidine and phenazopyridine on confluent MRC-9 cells does not show appreciable toxicity – 16% untreated vs. 17.6% – when cells received maximal pentamidine and phenazopyridine treatment. The values of percent inhibition were derived from multiple experiments. Negative numbers reflect noise.

**Table 7: percent inhibition of viability of human fibroblast MRC-9 cells**

| | | Pentamidine (μM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.840 | 0.420 | 0.210 | 0.105 | 0.053 | 0.026 | 0.013 | 0.007 | 0.003 | 0 |
| Phenazopyridine (μM) | 40.00 | 17.6 | 20.0 | 21.2 | 15.6 | 18.4 | 3.9 | 22.1 | 12.4 | 10.2 | 2.8 |
| | 20.00 | 6.0 | 28.6 | 3.5 | -2.7 | 28.3 | -2.3 | -1.4 | 16.3 | 1.7 | -0.8 |
| | 10.00 | 8.5 | 6.1 | 14.6 | 3.6 | 23.9 | 31.9 | 18.0 | 3.1 | 5.8 | 9.1 |
| | 5.00 | 19.2 | 16.3 | 17.0 | 16.3 | 6.5 | 7.3 | 19.7 | 0.7 | 1.9 | 5.0 |
| | 2.50 | 25.4 | 21.1 | 39.1 | 13.3 | 19.5 | 31.3 | 25.0 | 4.9 | 12.8 | 16.4 |
| | 1.25 | 32.2 | 44.7 | 20.9 | 26.7 | 26.6 | 27.3 | 20.0 | 15.6 | 34.8 | 9.8 |
| | 0.63 | 4.7 | 4.2 | 9.1 | 9.4 | 13.2 | 6.6 | 6.8 | 2.0 | 0.6 | -1.6 |
| | 0.31 | 15.8 | 18.7 | 6.9 | 14.7 | 5.6 | 13.1 | 7.9 | 21.1 | 16.8 | 7.3 |
| | 0.16 | 6.8 | 8.5 | 3.7 | 11.8 | 5.9 | 16.0 | 15.2 | 1.1 | -5.9 | 1.1 |
| | 0 | 12.4 | 13.1 | 11.8 | 2.1 | 15.0 | 34.6 | 13.6 | 14.8 | 4.4 | 16.0 |

## Example 6: pentamidine and pentolinium combinations for treatment of candidosis

Using the methods described below, we tested the ability of the combination of various concentrations of pentamidine plus pentolinium to inhibit the proliferation of numerous yeast strains using particle density as an indicator of cell number. In addition, we performed colony-forming assays to test the relative biocidal activity of specific concentrations of pentamidine and pentolinium. Finally, as a preliminary measure of the toxicity of these

combinations to mammalian cells, we added the pentamidine/pentolinium combination to cells in culture and assayed for viable cells using the reduction of Alamar Blue.

The graphs in Figs. 3-5 depict the biocidal activities of the pentamidine and pentolinium combination in *C. albicans*. Suspension cultures of *C. albicans* were grown at a starting density of 500 colony-forming units (CFUs) per mL in 10 mL RPMI media supplemented with 2% glucose. Cultures were treated with pentamidine, pentolinium, AmpB, or pentamidine plus pentolinium at the indicated concentrations. As a control, a suspension culture of *C. albicans* cells was left untreated. Cultures were incubated for 24 hours at 32°C with shaking at 250 rpm. Following this 24-hour incubation, the absorbance of each culture was measured. One thousand particles from each culture was plated on sabouraud agar plates with no supplementary compounds and incubated overnight at 32°C. Following the overnight incubation, the actual number of colonies growing on each plate was counted. Results indicate that treatment of *C. albicans* with either single agent suppresses growth, however removal of compounds by plating results in significant colony growth. In contrast, treatment with pentamidine and pentolinium not only repressed growth in suspension, *C. albicans* treated with pentamidine and pentolinium showed dramatically repressed colony growth following plating when the combination has been removed.

Tables 8 and 9 demonstrate the antiproliferative effects of combining pentamidine-isethionate and pentolinium-ditartrate to a clinical isolate of the opportunistic yeast, *Candida albicans*.

**Table 8: percent inhibition of proliferation of *Candida albicans* strain 17**

| | Pentolinium (µM) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 7.43 | 3.72 | 1.86 | 0.93 | 0.46 | 0.23 | 0.12 | 0.06 | 0.03 | 0.00 |
| Pentamidine (µM) | 3.37 | 88.55 | 88.88 | 86.31 | 87.51 | 87.58 | 88.43 | 88.16 | 89.19 | 87.74 | 86.28 |
| | 1.69 | 85.05 | 84.08 | 85.21 | 84.99 | 85.09 | 84.45 | 82.95 | 82.78 | 87.27 | 87.43 |
| | 0.84 | 82.32 | 84.49 | 83.60 | 85.98 | 84.41 | 84.98 | 83.28 | 84.79 | 84.66 | 83.27 |
| | 0.42 | 88.91 | 87.81 | 87.55 | 87.73 | 86.66 | 87.89 | 88.22 | 88.62 | 88.98 | 87.42 |
| | 0.21 | 93.72 | 93.22 | 93.82 | 94.05 | 93.71 | 93.81 | 93.02 | 93.16 | 92.64 | 93.41 |
| | 0.11 | 94.88 | 95.01 | 95.05 | 94.88 | 94.84 | 95.01 | 94.81 | 94.78 | 94.86 | 94.72 |
| | 0.05 | 94.46 | 94.70 | 94.42 | 94.43 | 93.85 | 91.07 | 90.74 | 92.75 | 91.56 | 88.42 |
| | 0.03 | 91.44 | 92.54 | 88.83 | 89.13 | 82.80 | 84.30 | 68.75 | 66.55 | 70.22 | 51.03 |
| | 0.01 | 86.60 | 89.65 | 82.82 | 77.46 | 71.28 | 66.26 | 38.75 | 43.32 | 20.93 | 26.70 |
| | 0.00 | 75.74 | 75.57 | 69.72 | 61.35 | 54.12 | 34.02 | 14.36 | 7.51 | 1.34 | -4.27 |

**Table 9: percent inhibition of proliferation of *Candida albicans* strain 17**

| | Pentolinium (µM) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 7.43 | 3.72 | 1.86 | 0.93 | 0.46 | 0.23 | 0.12 | 0.06 | 0.03 | 0.00 |
| Pentamidine (µM) | 0.4200 | 84.1 | 81.5 | 69.7 | 65.5 | 68.3 | 63.7 | 67.3 | 70.9 | 73.1 | 67.2 |
| | 0.2100 | 95.2 | 94.6 | 83.7 | 76.1 | 65.1 | 68.6 | 75.6 | 68.3 | 77.0 | 75.6 |
| | 0.1050 | 96.7 | 95.5 | 90.8 | 88.8 | 65.5 | 73.9 | 72.1 | 72.1 | 76.3 | 72.5 |
| | 0.0525 | 97.0 | 96.1 | 94.3 | 96.4 | 93.0 | 90.2 | 86.5 | 87.8 | 93.4 | 89.0 |
| | 0.0263 | 95.9 | 95.1 | 94.7 | 86.3 | 47.7 | 59.6 | 74.5 | 76.2 | 65.3 | 70.7 |
| | 0.0131 | 92.5 | 89.8 | 79.8 | 44.8 | 33.2 | 40.2 | -3.3 | 8.4 | 5.7 | 6.3 |
| | 0.0066 | 89.5 | 77.3 | 70.5 | 29.0 | 5.0 | -3.3 | -1.3 | -1.3 | -2.2 | -3.6 |
| | 0.0033 | 82.4 | 78.1 | 53.8 | 30.6 | 12.6 | -1.0 | -3.8 | -2.7 | -3.2 | -4.0 |
| | 0.0016 | 80.2 | 84.6 | 69.7 | 42.7 | 10.1 | -1.4 | -2.9 | -3.2 | -3.1 | -3.1 |
| | 0.0000 | 85.2 | 80.0 | 82.9 | 49.8 | 24.8 | -2.5 | -2.1 | -3.4 | -2.8 | -2.9 |

**Example 7: pentamidine and pentolinium combination treatment of *Candida spp.***

The effects of pentamidine and pentolinium were further tested on additional *Candida albicans* strains (Table 10).

**Table 10: Percent inhibition of proliferation of triazole-resistant *Candida albicans* strain MYA 573 cells**

| | | Pentolinium (µM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 7.43 | 3.72 | 1.86 | 0.93 | 0.46 | 0.23 | 0.12 | 0.06 | 0.03 | 0.00 |
| Pentamidine (µM) | 0.8400 | 73.7 | 76.7 | 74.1 | 68.6 | 73.0 | 75.9 | 71.4 | 74.1 | 73.8 | 73.5 |
| | 0.4200 | 36.4 | 36.1 | 43.2 | 40.7 | 43.0 | 35.6 | 37.8 | 35.6 | 41.5 | 39.7 |
| | 0.2100 | 24.2 | 22.5 | 26.0 | 19.4 | 14.7 | 18.5 | 21.5 | 24.5 | 19.1 | 23.8 |
| | 0.1050 | 16.5 | 12.7 | 17.8 | 14.6 | 16.2 | 20.1 | 14.6 | 18.0 | 11.4 | 10.6 |
| | 0.0525 | 13.2 | 12.1 | 14.2 | 12.8 | 11.2 | 10.0 | 7.6 | 0.4 | 4.3 | -1.5 |
| | 0.0263 | 9.1 | 5.8 | -1.7 | 12.1 | 2.6 | 7.3 | 6.8 | 2.5 | -0.3 | 8.7 |
| | 0.0131 | 3.2 | 4.3 | 3.2 | 11.9 | 1.5 | 8.4 | 7.8 | -3.2 | -9.9 | -0.2 |
| | 0.0066 | -2.1 | -2.1 | 5.2 | 2.1 | 8.1 | -0.1 | -3.0 | -1.7 | -3.2 | -1.9 |
| | 0.0033 | 1.5 | 4.4 | 2.7 | 7.2 | 4.2 | -3.4 | -4.1 | -6.4 | -2.3 | -4.3 |
| | 0.0000 | 9.9 | 5.1 | 1.2 | -2.1 | 1.6 | 0.9 | 5.3 | 3.7 | -4.1 | -5.3 |

**Example 8: toxicity of pentamidine and pentolinium combination in treatment of human cells**

As a preliminary measure of toxicity, we studied the effects of the combination of pentamidine and pentolinium in confluent human MRC-9 fibroblast cells. MRC-9 cells are non-transformed, diploid cells, which exhibit contact-inhibited cell growth characteristics. As is shown in Table 11, below, the combination of pentamidine and pentolinium on confluent MRC-9 cells does not show appreciable toxicity.

**Table 11: percent inhibition of viability of human fibroblast MRC-9 cells**

| | | Pentamidine (μM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.840 | 0.420 | 0.210 | 0.105 | 0.053 | 0.026 | 0.013 | 0.007 | 0.003 | 0 |
| Pentolinium (μM) | 7.43 | 15.4 | 15.6 | 19.9 | 13.2 | 11.8 | 10.2 | 4.6 | 6.5 | 4.1 | 3.3 |
| | 3.72 | -1.9 | 24.4 | 8.0 | 41.3 | 10.6 | 16.8 | 7.0 | 2.8 | -1.8 | 1.9 |
| | 1.86 | 28.3 | 34.0 | 44.4 | 19.2 | 36.3 | 35.1 | 22.0 | 21.1 | 31.9 | 15.3 |
| | 0.93 | 6.5 | 16.7 | 33.2 | 26.2 | 31.6 | 17.7 | 15.8 | 12.0 | 12.5 | 29.9 |
| | 0.46 | 30.3 | 30.9 | 43.7 | 45.8 | 39.0 | 23.8 | 35.4 | 40.5 | 17.8 | 34.0 |
| | 0.23 | 17.3 | 49.1 | 32.6 | 43.1 | 42.1 | 42.9 | 40.5 | 16.6 | 14.7 | 22.4 |
| | 0.12 | 20.0 | 22.7 | 45.0 | 39.7 | 21.1 | 34.0 | 32.9 | 23.2 | 24.6 | 17.9 |
| | 0.06 | 13.5 | 18.8 | 32.7 | 20.2 | 33.4 | 25.0 | 29.6 | 20.1 | 26.1 | 7.1 |
| | 0.03 | 18.4 | 23.7 | 23.7 | 11.1 | 25.7 | 22.5 | 21.1 | 11.7 | 19.4 | 10.9 |
| | 0.00 | 13.3 | 21.4 | 22.0 | 23.5 | 22.7 | 15.1 | 12.5 | 9.1 | 10.5 | 4.9 |

**Experimental procedures**

The foregoing results were obtained with the following materials and methods.

**Fungal strains**

Two clinical *Candida albicans* isolates (one triazole-resistant strain and one triazole-susceptible strain) were obtained from the culture collection if the Seattle Biomedical Research Institute (Seattle, WA). Triazole-resistant *C. albicans* was obtained from the American Type Culture Collection (ATCC MYA-573). Yeast cultures were stored in 15% glycerol at –70°C. Isolates were cultured in growth medium (RPMI; 2% glucose without $NaCO_2$ and phenol red, buffered with 0.165 M MOPS to pH 7.0) at 35°C for 24 hours prior to *in vitro* susceptibility testing. In some experiments, all strains were included, while in others only the *C. albicans* strains were used. All media reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

The *Aspergillus fumigatus* strain was obtained from the American Type Culture Collection ATCC 46645. *Aspergillus* cultures were stored in 15% glycerol at –70°C. Isolates were cultured on Sabouraud glucose (SAB) agar plates at 37°C for 5 to7 days. Condidia were collected by adding 5 to 10 mL of

sterile saline containing 0.05% TWEEN 20™ to the plates and loosening condidia with a swab. Using a pipette, condidia were collected suspension and place in a sterile bottle. Repeat for all plates. After harvesting the suspension was filtered through gauze into a clean, sterile bottle. The turbidity of the supernatants was measured spectrophometrically at 530 nm and the transmission adjusted to 80-82% with PBS (with 0.05% TWEEN 20 ™) to yield an initial suspension of 1 x $10^6$ CFU/mL.

The *Aspergillus* suspension was prepared in the above manner and diluted in growth medium to yield a final inoculum concentration of approximately 4 x $10^4$ cells/mL. Forty microlitres of the inoculum were used to inoculate each well, resulting in a final concentration of 2 x $10^4$ cells/mL. Drug-free controls were included on each plate. Plates were incubated for 24 hours at 35°C. The amount of cell growth was determined by microscopic analysis.

**Compounds and compound preparation**

The following compounds were used: pentamidine isethionate, pentolinium ditartrate, phenazopyridine hydrochloride, and amphotericin B (all from Sigma Chemical Co.). In these experiments, amphotericin B was used as a positive control for antifungal activity. Stock solutions of each compound were prepared in DMSO and stored at –20°C. Prior to use, stock solutions were diluted in growth medium to produce 10X solutions. 10X pentamidine (0-7 μL) and 10X of one of pentolinium and phenazopyridine (0-7 μL) were then plated in 45 μL of growth medium in 384-well microtiter plates to form a matrix of compound combinations.

**Anti-proliferation assay**

All antifungal susceptibility testing was performed according to document M-27A as published by the National Committee for Clinical Laboratory Standards (Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts (1997), Wayne, PA). Briefly, yeast inocula from overnight cultures were standardized to a turbidity equivalent to a 0.5 McFarland Standard using a spectrophotometer at 530 nm, giving rise to a stock solution of 1 x $10^6$ cells/mL. Each yeast suspension was further diluted in growth medium to yield a final inoculum concentration of approximately 4 x $10^3$ cells/mL. Forty microlitres of the inoculum were used to inoculate each well, resulting in a final concentration of 2 x $10^3$ cells/mL. Drug-free controls were included on each plate. Plates were incubated for 16 hours at 32°C. The amount of cell growth was determined by particle density as read on a Nephelometer (BMG Lab Technologies; Durham, North Carolina).

**Colony-forming assay**

Suspension cultures of C. *albicans* were grown at a starting density of 500 colony-forming units (CFUs) per mL in 10 mL RPMI media supplemented with 2% glucose. Cultures were treated with pentamidine at a concentration of 0.2-2.6 μM, pentolinium at 3 μM, phenazopyridine at 5 μM, amphotericin B (AmpB) at 2 μM or a combination of pentamidine and pentolinium at concentrations of 0.2-2.6 μM and 3 μM, respectively, or of pentamidine and phenazopyridine at concentrations of 0.5 μM and 5 μM, respectively. As a control, a suspension culture of C. *albicans* was left untreated. Cultures were incubated for 24 hours at 32°C with shaking at 250 rpm. Following this 24-hour incubation, the absorbance of each culture was measured. Cultures were centrifuged at 3000 rpm for five minutes, the supernatant was removed, and

pellets were resuspended in fresh media. One thousand particles from each culture were plated on sabouraud agar plates with no supplementary compounds and incubated overnight at 32°C. Following the overnight incubation, the actual number of colonies growing on each plate was counted.

**Cellular toxicity assay**

An examination of the toxicity of the pentamidine/pentolinium and pentamidine/phenazopyridine combinations on confluent, non-proliferating cells was performed. The MRC-9 cell line (ATCC: CCL-212) is a human fibroblast line cultured in Minimum Essential Media (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotic. 384-well tissue culture plates were seeded with 5000 MRC-9 cells per well (125,000 cells /mL). These assay plates were incubated for 18 hours at 37°C in a humidified atmosphere containing 5% $CO_2$. At 18-hour time points, dilutions of compounds were prepared as described above and added directly to assay plates. Assay plates were incubated for an additional 24 hours at 37°C. Following a 24-hour incubation period, Alamar Blue was added to each well to a final concentration of 5%. Alamar Blue is metabolically reduced by the cell mitochondria to produce a fluorescent product, the amount of which (referred to as the "RFU") being proportional to the cell number. After Alamar Blue addition, plates were incubated for an additional three hours. Subsequently, the RFU of each well was determined fluorometrically, using a microplate reader (Wallac, model number: 1420-0142; Perkin Elmer, Gaithersburg, MD) equipped with a 540 nm excitation filter and a 590 nm emission filter.

**Determination of inhibition or toxicity**

The determination of toxicity was determined using the following formula: [(RFU control-RFU test)/RFU control] * 100 = percent inhibition.

Percent inhibition was determined using the following formula: [(particle density control - particle density test)/particle density control] * 100 = percent inhibition.

## Other Embodiments

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in cellular and molecular biology, pharmacology, endocrinology, or related fields are intended to be within the scope of the invention.

What is claimed is:

## Claims

1. A method for treating a patient who has a fungal infection, or inhibiting the development of a fungal infection in a patient who is at risk for developing a fungal infection, said method comprising administering to said patient (i) an aromatic diamidine or an analog thereof or a compound of formula (I); and (ii) an aminopyridine, a quaternary ammonium compound, or a compound of formula (II) or (III), wherein the two compounds are administered simultaneously or within 10 days of each other, in amounts sufficient to treat or inhibit the development of a fungal infection in said patient.

2. The method of claim 1, wherein said aromatic diamidine is selected from the group consisting of pentamidine, propamidine, butamidine, heptamidine, nonamidine, stilbamidine, hydroxystilbamidine, diminazene, benzamidine,
4,4'- (pentamethylenedioxy) di-, dihydrochloride, phenamidine, dibrompropamidine, 1,3-bis(4-amidino-2-methoxyphenoxy)propane, netropsin, distamycin, and phenamidine.

3. The method of claim 1, wherein said aminopyridine is phenazopyridine, 4-aminopyridine, 3,4-diaminopyridine, 2,5-diamino-4-methylpyridine, 2,3,6-triaminopyridine, 2,4,6-triaminopyridine, or 2,6-diaminopyridine.

4. The method of claim 1, wherein said quaternary ammonium compound is pentolinium, hexamethonium, pentamethonium, tetramethylammonium, tetraethylammonium, trimethaphan, or chlorisondamine.

5. The method of claim 1, wherein said aromatic diamidine is pentamidine and said aminopyridine is phenazopyridine.

6. The method of claim 1, wherein said aromatic diamidine is pentamidine and said quaternary ammonium compound is pentolinium.

7. The method of any of claims 1-6, wherein the two compounds are administered within five days of each other.

8. The method of claim 7, wherein the two compounds are administered within 24 hours of each other.

9. The method of claim 8, wherein the two compounds are administered within one hour of each other.

10. The method of claim 9, wherein the two compounds are administered simultaneously.

11. The method of any one of claims 1-10, wherein said patient has or is at risk of developing tinea capitis, tinea corporis, tinea pedis, tinea barbae, tinea cruris, tinea versicolor, onychomycosis, perionychomycosis, pityriasis versicolor, tinea unguium, oral thrush, vaginal candidosis, respiratory tract candidosis, biliary candidosis, eosophageal candidosis, urinary tract candidosis, systemic candidosis, mucocutaneous candidosis, mycetoma, cryptococcosis, aspergillosis, mucormycosis, chromoblastomycosis, paracoccidioidomycosis, North American blastomycosis, histoplasmosis, coccidioidomycosis, or sporotrichosis.

12. The method of any one of claims 1-10, wherein said fungal infection is an infection of *Candida albicans*, *Candida krusei*, *Candida glabrata*, *Cryptococcus neoformans* or *Aspergillus spp*.

13. The method of claim 1, wherein each of the compounds is, independently, administered to said patient by intravenous, rectal, oral, topical, intravaginal, ophthalmic, or inhalation administration.

14. The method of claim 1, wherein said aromatic diamidine or compound of formula (I) is administered in an amount between 0.001 and 2000 mg per day.

15. The method of claim 1, wherein said aminopyridine is administered in an amount between 0.001 mg per day per day and 2400 mg per day.

16. The method of claim 1, wherein said quaternary ammonium compound is administered in an amount between 0.5 and 500 mg per day.

17. The method of claim 1, wherein said aromatic diamidine is pentamidine, said aminopyridine is phenazopyridine, and said pentamidine and phenazopyridine are administered at a ratio of 2-1000 parts by molar ratio of phenazopyridine to one part by molar ratio of pentamidine.

18. The method of claim 1, wherein said aromatic diamidine is pentamidine, said quaternary ammonium compound is pentolinium, and said pentamidine and pentolinium are administered at a ratio of 2-1000 parts by molar ratio of pentolinium to one part by molar ratio of pentamidine.

19. A composition comprising (i) an aromatic diamidine or analog thereof or a compound of formula (I); and (ii) an aminopyridine, a quaternary ammonium compound, or a compound of formula (II) or (III), wherein the two compounds are present in amounts that together inhibit or reduce fungal growth.

20. The composition of claim 19, wherein said aromatic diamidine is pentamidine, propamidine, butamidine, heptamidine, nonamidine, stilbamidine, hydroxystilbamidine, diminazene, benzamidine, 4,4'- (pentamethylenedioxy) di-, dihydrochloride, phenamidine, dibrompropamidine, 1,3-bis(4-amidino-2-methoxyphenoxy)propane, netropsin, distamycin, or phenamidine.

21. The composition of claim 19, wherein said aminopyridine is phenazopyridine, 4-amino-pyridine, 3,4-diaminopyridine, 2,5-diamino-4-methylpyridine, 2,3,6-triaminopyridine, 2,4,6-triaminopyridine, or 2,6-diaminopyridine.

22. The composition of claim 19, wherein said quaternary ammonium compound is pentolinium, hexamethonium, pentamethonium, tetramethylammonium, tetraethylammonium, trimethaphan, or chlorisondamine.

23. A pharmaceutical pack comprising (i) an aromatic diamidine or analog thereof or a compound of formula (I); and (ii) an aminopyridine, a quaternary ammonium compound, or a compound of formula (II) or (III).

24. The pharmaceutical pack of claim 23, wherein said aromatic diamidine is pentamidine, propamidine, butamidine, heptamidine, nonamidine, stilbamidine, hydroxystilbamidine, diminazene, benzamidine, 4,4'-(pentamethylenedioxy) di-, dihydrochloride, phenamidine, dibrompropamidine, 1,3-bis(4-amidino-2-methoxyphenoxy)propane, netropsin, distamycin, or phenamidine.

25. The pharmaceutical pack of claim 23, wherein said aminopyridine is phenazopyridine, 4-amino-pyridine, 3,4-diaminopyridine, 2,5-diamino-4-methylpyridine, 2,3,6-triaminopyridine, 2,4,6-triaminopyridine, or 2,6-diaminopyridine.

26. The pharmaceutical pack of claim 23, wherein said quaternary ammonium compound is pentolinium, hexamethonium, pentamethonium, tetramethylammonium, tetraethylammonium, trimethaphan, or chlorisondamine.

27. The pharmaceutical pack of any one of claims 23-26, wherein the two compounds are formulated separately and in individual dosage amounts.

28. The pharmaceutical pack of any one of claims 23-26, wherein the two compounds are formulated together.

29. A method of preventing, stabilizing, or inhibiting the growth of fungal cells, said method comprising contacting said fungal cells with (i) an aromatic diamidine or analog thereof or a compound of formula (I); and (ii) an aminopyridine, a quaternary ammonium compound, or a compound of formula (II) or (III) in amounts that, in combination, are sufficient to prevent, stabilize, or inhibit the growth of the fungal cells.

30. A method for preventing, stabilizing, or inhibiting the growth of fungal cells on a surface, said method comprising contacting said surface with (i) an aromatic diamidine or analog thereof or a compound of formula (I); and (ii) an aminopyridine, a quaternary ammonium compound, or a compound of formula (II) or (III) in amounts sufficient to prevent stabilize, or inhibit growth of said fungal cells.

31. The method of claim 30, wherein said surface is selected from the group of process equipment, water sanitation systems, cooking utensils, food preparation areas, and medical devices.

32. The method of claim 31, wherein said medical devices are selected from the group consisting of surgical tools, dental tools, dental appliances, orthodontic braces, dentures, stents, endoscopy equipment, surgical implants, prosthetic devices, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, and continuous ambulatory peritoneal dialysis catheters.

33. The method of claim 29 or 30, wherein said aromatic diamidine is pentamidine, propamidine, butamidine, heptamidine, nonamidine, stilbamidine, hydroxystilbamidine, diminazene, benzamidine, 4,4'- (pentamethylenedioxy) di-, dihydrochloride, phenamidine, dibrompropamidine, 1,3-bis(4-amidino-2-methoxyphenoxy)propane, netropsin, distamycin, or phenamidine.

34. The method of claim 29 or 30, wherein said aminopyridine is phenazopyridine, 4-amino-pyridine, 3,4-diaminopyridine, 2,5-diamino-4-methylpyridine, 2,3,6-triaminopyridine, 2,4,6-triaminopyridine, or 2,6-diaminopyridine.

35. The method of claim 29 or 30, wherein said quaternary ammonium compound is pentolinium, hexamethonium, pentamethonium, tetramethylammonium, tetraethylammonium, trimethaphan, or chlorisondamine.

36. A method for identifying combinations of compounds useful for treating a patient having a fungal infection, said method comprising the steps of:

(a) contacting fungal cells *in vitro* with (i) an aromatic diamidine, a quaternary ammonium compound and/or an aminopyridine, and (ii); a candidate compound; and

(b) determining whether the combination of said aromatic diamidine, a quaternary ammonium compound and/or an aminopyridine and said candidate compound reduces growth of said fungal cells relative to fungal cells contacted with said aromatic diamidine, a quaternary ammonium compound and/or an aminopyridine in the absence of said candidate compound, or fungal cells contacted with said candidate compound but not with said aromatic diamidine, a quaternary ammonium compound and/or an aminopyridine, wherein a decrease in fungal growth identifies said combination as a combination that is useful for treating a patient having a fungal infection.

37. A method for treating a patient who has a fungal infection, or inhibiting the development of a fungal infection in a patient who is at risk for developing a fungal infection, said method comprising administering to said patient (i) an antifungal agent and (ii) an aromatic diamidine, aminopyridine, or quaternary ammonium compound, or a compound of formula (I), (II), or (III), wherein the two compounds are administered simultaneously or within 10 days of each other, in amounts sufficient to treat or inhibit the development of a fungal infection in said patient.

38. The method of claim 37, wherein said antifungal agent is selected from the group consisting of: amphotericin B, fluconazole, nystatin, pimaricin, ketoconazole, miconazole, thiabendazole, emlkonazole, itraconazole, ravuconazole, posaconazole, voriconazole, dapsone, griseofulvin, carbol-fuchsin, clotrimzole, econazole, haloprogin, mafenide, naftifine, oxiconazole, silver sulfadiazine, sulconazole, terbinafine, amorolfine, tioconazole, tolnaftate, undecylenic acid, butoconazle, gentian violet, terconazole, flucytosine, ciclopirox, caspofungin acetate, micafungin, and V-echinocandin (LY303366).

39. The method of claim 37 or 38, wherein said aromatic diamidine is selected from the group consisting of pentamidine, propamidine, butamidine, heptamidine, nonamidine, stilbamidine, hydroxystilbamidine, diminazene, benzamidine, 4,4'- (pentamethylenedioxy) di-, dihydrochloride, phenamidine, dibrompropamidine, 1,3-bis(4-amidino-2-methoxyphenoxy)propane, netropsin, distamycin, and phenamidine.

40. The method of claim 37 or 38, wherein said aminopyridine is phenazopyridine, 4-amino-pyridine, 3,4-diaminopyridine, 2,5-diamino-4-methylpyridine, 2,3,6-triaminopyridine, 2,4,6-triaminopyridine, or 2,6-diaminopyridine.

41. The method of claim 37 or 38, wherein said quaternary ammonium compound is pentolinium, hexamethonium, pentamethonium, tetramethylammonium, tetraethylammonium, trimethaphan, or chlorisondamine.

FIG. 1

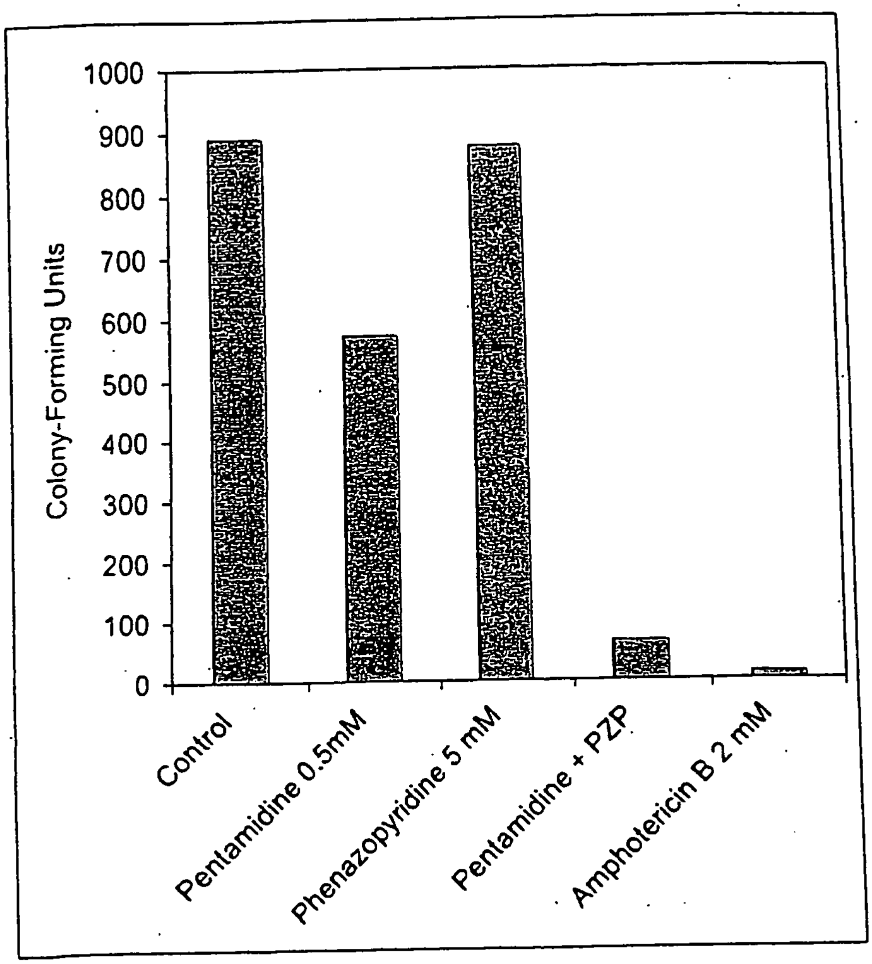

1000
900
800
700
600
500
400
300
200
100
0
Colony-Forming Units
Control
Pentamidine 0.5mM
Phenazopyridine 5 mM
Pentamidine + PZP
Amphotericin B 2 mM

FIG. 2

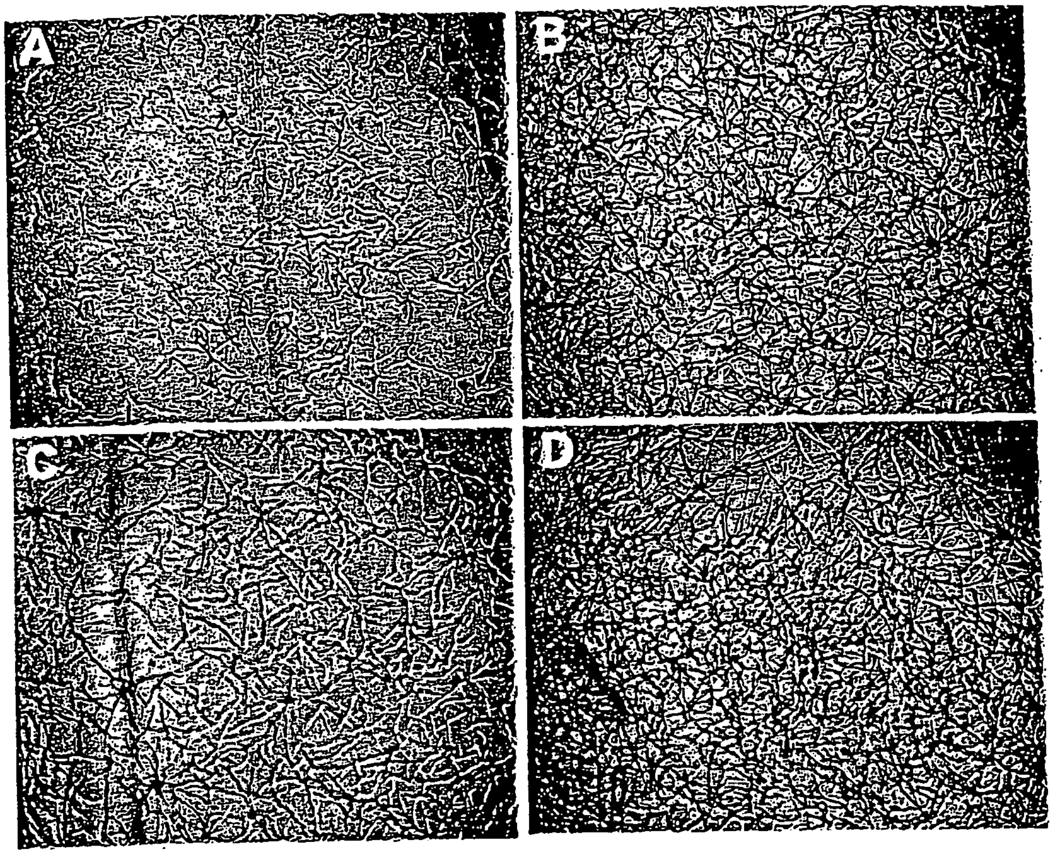


FIG. 3

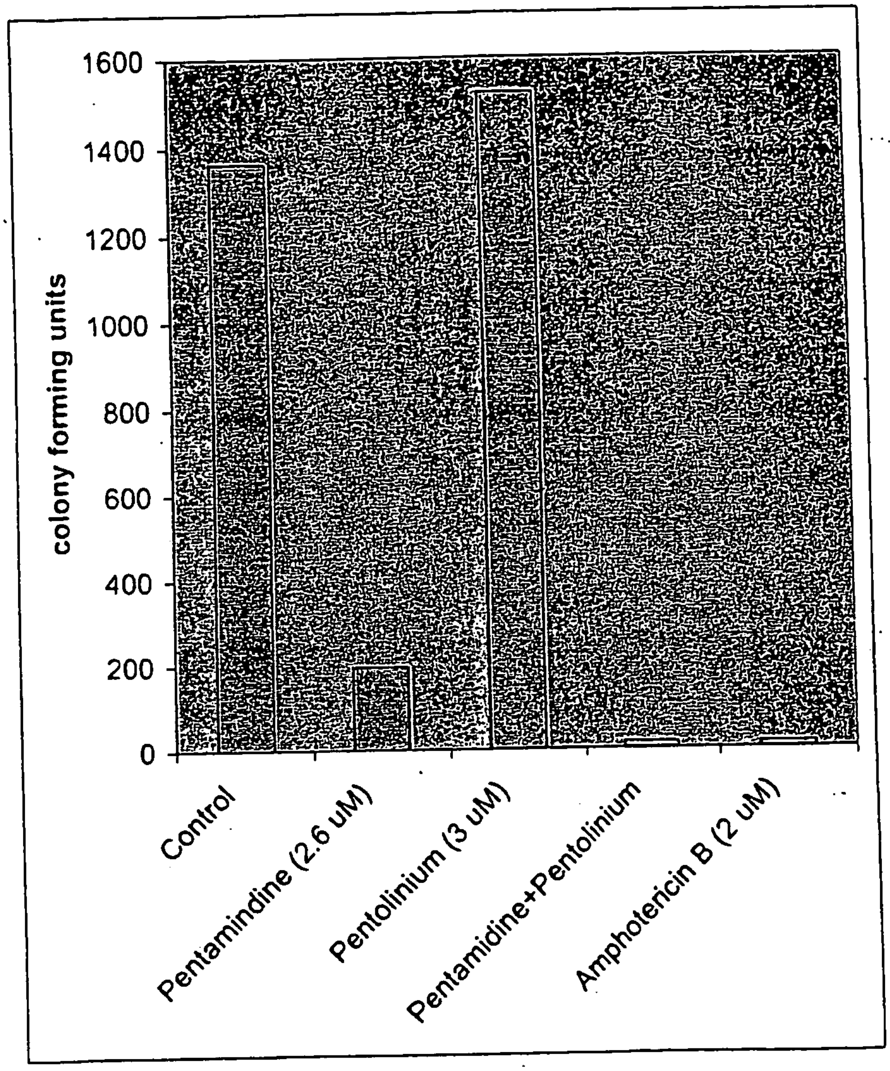

colony forming units
1600
1400
1200
1000
800
600
400
200
0
Control
Pentamindine (2.6 uM)
Pentolinium (3 uM)
Pentamidine+Pentolinium
Amphotericin B (2 uM)

FIG. 4

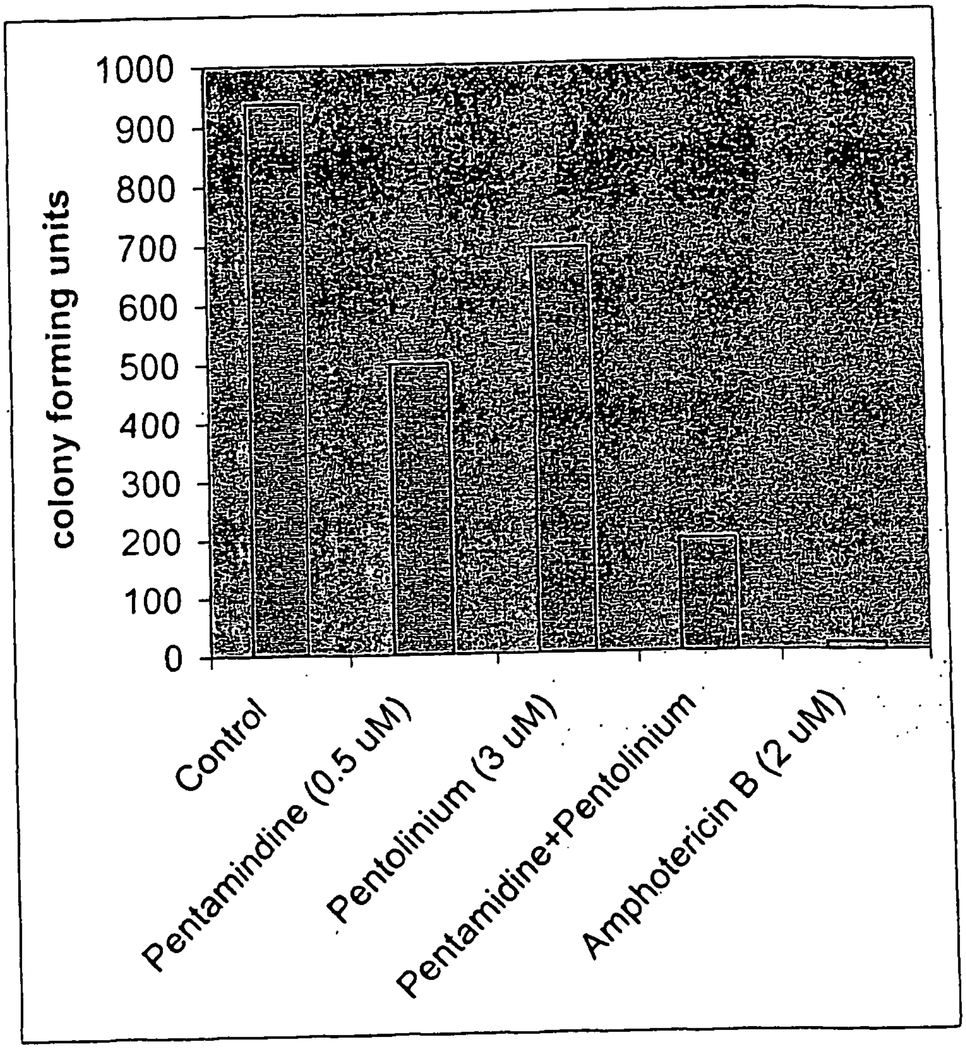
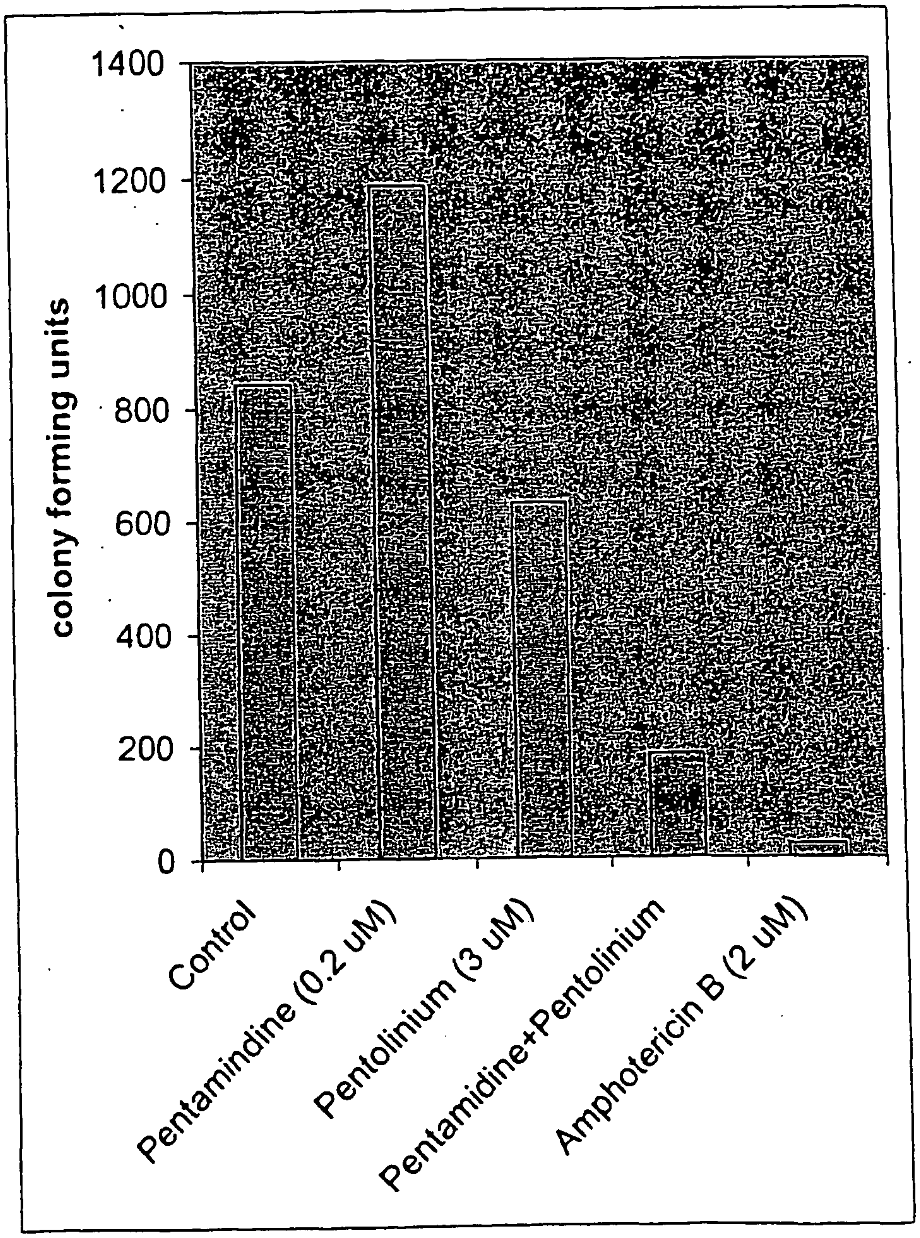

1000
900
800
700
600
500
400
300
200
100
0
colony forming units
Control
Pentamindine (0.5 uM)
Pentolinium (3 uM)
Pentamidine+Pentolinium
Amphotericin B (2 uM)

FIG. 5



1400
1200
1000
800
600
400
200
0
colony forming units
Control
Pentamidine (0.2 uM)
Pentolinium (3 uM)
Pentamidine+Pentolinium
Amphotericin B (2 uM)